Austalasian Neuroscience Society Annual Scientific Meeting 2016

Abstracts for Poster Presentations

Posters are listed by Poster Number

Poster 1 – Tuesday 6th December

CAV-1 ABLATION PROTECTS AGAINST LOSS OF INNER RETINAL FUNCTION CAUSED BY PTPN11 OVEREXPRESSION.

Mojdeh Abbasi1, Vivek Gupta1, Nitin Chitranshi1, Yogita Dheer1, Anita Turner1, Roshana Vanderwall1, Stuart L. Graham1,2.

1.Macquarie University, Sydney, NSW, Australia.
2.University of Sydney, Save Sight Institute, Sydney, NSW, Australia.

Brain derived neurotrophic factor (BDNF) and its receptor tropomysin receptor kinaseB (TrkB) play a protective role in survival of retinal ganglion cells (RGCs). PTPN11 is a ubiquitously expressed tyrosine phosphatase that regulates TrkB and its activation is mediated through its interactions with the adapter protein caveolin (Cav-1). This study examines the effects of Cav-1 in PTPN11 mediated inner retinal changes using Cav-1 ablation in an animal model.
PTPN11 was overexpressed in mice retina (n=38) through intravitreal injection of recombinant adeno-associated virus vector (AAV-PTPN11). Wildtype (WT), Cav-1 null (KO) and Cav-1 heterozygous (Het) mice were used and for each animal one eye was injected with AAV-PTPN11 whereas the contralateral eye was used as control. Retinal function was assessed by electroretinogram (ERG) and scotopic threshold response (STR) recordings. Optical coherence tomography (OCT) and H&E staining were used to evaluate retinal structural changes.
WT and Het mice retinas overexpressing PTPN11 exhibited a significantly reduced STR amplitude compared to controls ($p<0.003$). Although KO group demonstrated a lower STR amplitude compared to controls, the reduction was not statistically significant. OCT imaging showed no significant alterations between the retinal thickness among mentioned groups. However, Microscopy revealed a significantly lower number of RGCs in the WT/Het following PTPN11 upregulation when compared to GFP controls ($p<0.005$).
Our results suggest a novel role of Cav-1 in mediating PTPN11 actions in retina. Loss of Cav-1 exerts a protective effect particularly on inner retinal function and structure. Future studies will help establish the pathophysiological cross talk between these two proteins.

Poster 2 – Monday 5th December

RECIPROCAL CONTROL OF DRINKING BEHAVIOR BY MEDIAN PREOPTIC NEURONS IN MICE.

Abbott S.B.G. [1,2,3], Saper, C.B. [1]

1. Department of Neurology, Beth Israel-Deaconess Medical Center - Harvard Medical School, Boston, 2. The Heart Research Institute, Sydney, 3. Department of Physiology, University of Sydney

Stimulation of glutamatergic neurons in the subfornical organ drives drinking behavior, but the brain targets that mediate this response are not known. The densest target of subfornical axons is the anterior tip of the third ventricle, containing the median preoptic nucleus (MnPO) and organum vasculosum of the lamina terminalis (OVLT), a region that has also been implicated in fluid and electrolyte management. The neurochemical composition of this region is complex, containing both GABAergic and glutamatergic neurons, but the possible roles of these neurons in drinking response have not been addressed. In mice, we show that optogenetic stimulation of glutamatergic neurons in MnPO/OVLT drives voracious water consumption, and that optogenetic stimulation of GABAergic neurons in the same region selectively reduces water consumption. Both populations of neurons have extensive projections to overlapping regions of the thalamus, hypothalamus and hind brain that are much more extensive than those from the subfornical organ, suggesting that the MnPO/OVLT serves as a key link in regulating drinking responses

Poster 3 – Tuesday 6th December

A NOVEL ROLE FOR STING IN NEUROINFLAMMATION AFTER TRAUMATIC BRAIN INJURY.

Abdullah A [1] , Zhang M [1] , Frugier TJ [1] , Taylor JM [1] , Crack PJ [1] .

[1] Department of Pharmacology & Therapeutics , University of Melbourne , VIC, Australia

Traumatic brain injury (TBI) represents a major cause of disability and death worldwide with sustained neuro-inflammation a key driver of cellular damage. STING-induced type-1 interferon (IFN) signaling is known to modulate the innate immune response in the periphery, however its role in the CNS remains unclear. We previously identified the type-1 IFN pathway as a key mediator of neuro-inflammation and neuronal cell death in TBI (Karve *et al*, 2016). This study investigated the role of STING in modulating the type-1 IFN mediated neuroinflammatory response following TBI. WT and STING-/- mice (male, n=6) were subjected to controlled cortical impact (CCI) injury and brains removed 2h or 24h for QPCR, western and immunohistochemical analysis. QPCR identified increased STING expression (4.45 $\pm$ 0.93 fold; $p<0.05$) in WT mice at 24h that was confirmed by western blot and immunohistochemistry. Elevated TNF-α, IL-6, i-NOS and IFN-β levels were also detected in the WT mice. Significantly, pro-inflammatory gene expression was suppressed in the STING-/- mice with a smaller infarct volume identified at 24h (WT; 4.16$\pm$ 0.27mm^3, STING-/- ; 3.20$\pm$0.17 mm^3; $p< 0.05$) as assessed by triphenyl tetrazolium

chloride (TTC) staining. Supporting a role for STING in human TBI, a significant upregulation in STING expression (2.25±0.50 fold; p< 0.0001) was detected in late trauma human brain samples as compared to the control group. These studies have identified STING as a novel mediator of neuroinflammation in TBI and therefore a potential new therapeutic target.

Poster 4 – Monday 5th December

ALTERED AMPA RECEPTOR EXPRESSION IN CORTICAL INHIBITORY INTERNEURONS OF THE EPILEPTIC STARGAZER MUTANT MOUSE

Adotevi NK, Leitch B

Department of Anatomy, Brain Health Research Centre, University of Otago, Dunedin, New Zealand.

Absence seizures arise from disturbances within the corticothalamocortical network, however the precise cellular and molecular mechanisms underlying seizure generation in patients from different genetic backgrounds are not fully understood. While recent experimental evidence suggests that changes in cortical inhibitory microcircuits may contribute to the generation of the hallmark spike-wave discharges, it is still unclear if these aberrations are a result of dysfunctional inhibitory interneurons due to compromised excitatory input, and/or whether differences in interneuron number also contribute. The stargazer mutant mouse, an established model of absence epilepsy, presents with a genetic deficit in stargazin, an auxillary protein which traffics glutamatergic AMPA receptors (AMPARs) to synapses. In the cortex, it is predominantly expressed in parvalbumin-positive (PV^+) inhibitory interneurons. Hence, the aim of this study was to examine changes in the expression of AMPAR subunits' GluA1-4 in the cortex, which could potentially alter excitatory inputs onto feed-forward PV^+ neurons, using western blotting and immunofluorescence confocal microscopy. We also analyzed PV^+ neuron density to identify any changes that could additionally compromise cortical inhibition. Our results reveal that while there are no differences in cortical PV^+ interneuron number, there are significant neuron-specific alterations in AMPAR expression in stargazers compared to non-epileptic littermates. This altered expression of AMPARs in PV^+ neurons could impair their feed-forward inhibitory output, ultimately altering cortical network oscillations and contributing to seizure generation. Elucidating cell and synapse specific changes in AMPAR expression in the cortex could be crucial to the development of targeted and effective therapies for treatment of absence seizures

Poster 5 – Tuesday 6th December

IN SILICO AND IN VITRO STUDIES OF THE NEUROPROTECTIVE EFFECTS OF ASTAXANTHIN AND FUCOXANTHIN AGAINST AMYLOID BETA (AB1-42) TOXICITY

Mousa Alghazwi[1, 2, 3], Wei Zhang[1, 2], Scott Smid[4]

[1]Flinders Centre for Marine Bioproducts Development (CMBD), [2]Department of Medical Biotechnology, School of Medicine, Flinders University, GPO Box 2100, Adelaide 5001, South Australia, Australia; [3]Ministry of Higher education in Saudi Arabia, King Faisal Hospital Street, Riyadh 11153, [4]Discipline of Pharmacology, School of Medicine, Faculty of Health Sciences, The University of Adelaide, South Australia, Australia

Senile plaques are a major hallmark of Alzheimer's disease, which is formed by the aggregation of amyloid beta (Aβ) protein. Marine-derived carotenoid compounds, such as astaxanthin and fucoxanthin have shown a spectrum of biological activities, including neuroprotection. In this study, we have used in silico modelling (CLC Drug Discovery Workbench) to investigate the interaction between these compounds and different structures of Aβ (monomer, pentamer, and fibril). The results showed that both astaxanthin and fucoxanthin exhibit a strong binding affinity towards Aβ. Subsequently, we investigated the neuroprotective effect of these compounds against Aβ-mediated toxicity in phaeochromocytoma (PC12) neuronal cells. Astaxanthin did not show any toxicity below 50 μM, while fucoxanthin was not toxic below 4 μM in the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cell viability assay. MTT assay was then used to determine the cell viability of Aβ1-42 (1 μM), alone and in combination with the two carotenoid compounds at different concentrations. Exposing PC12 cells to Aβ1-42 (1 μM) for 48 hrs resulted in approximately 30% toxicity. Both astaxanthin and fucoxanthin significantly inhibited the toxicity induced by Aβ1-42. Fucoxanthin showed greater neuroprotection (87-100% over 0.01-2 μM) than astaxanthin (88-96% over 0.1-50 μM). Astaxanthin demonstrated a greater anti-aggregative effect than fucoxanthin in the Thioflavin T (ThT) fluorometric assay of Aβ1-42 fibrillization. These results provide a promising avenue for further research into the neuroprotective role of marine-derived carotenoid compounds as novel dementia prevention or therapeutic strategies.

Poster 6 – Monday 5th December

ALTERED STEROIDOGENESIS IN FETAL BRAIN AND PLACENTA IN A DEVELOPMENTAL VITAMIN D (DVD) DEFICIENT RAT MODEL OF AUTISM SPECTRUM DISORDER (ASD)

Ali A, Cui X, Medley G and Eyles D.

Queensland Brain Institute, University of Queensland, Australia

Emerging evidence suggests that prenatal vitamin D deficiency is a risk factor for ASD. A recent study showed that neurosteroids including progesterone, 17-hydroxyprogesterone and androgens were significantly increased in the amniotic fluid (AF) of children who developed ASD. Aromatase is also reduced in post-mortem brains from autistic patients. Vitamin D regulates expression of several steroidogenic enzymes in vitro and be a risk-modifying factor for ASD itself. Here, we investigated the effects of DVD-deficiency on fetal steroidogenic

enzyme expression. Fetal steroidogenesis was examined in the brain, placentas and AF of male fetuses. Female Sprague-Dawley rats were offered vitamin D depleted diet for the period of 6-weeks before mating until embryonic day18. Male fetuses positioned between two neighbouring males (2M-males) or downstream to a male fetus (1M-males) were selected. Gene expression of steroidogenic enzymes and ASD-related genes were examined by Real-Time PCR in whole fetal brains and matching placentas. In brains, DVD-deficiency produced 16% and 26% reduction in aromatase of 1M and 2M-males respectively compared to similar positioned controls. Cyp21a1, foxp2 and the vitamin D activating enzyme cyp27b1 were significantly reduced in only 2M DVD-deficient brains. In placenta catechol-O-methyltransferase and cyp11a1 were down-regulated while cyp27b1 was up-regulated in both 1M and 2M DVD-deficient males. The alterations in aromatase and cyp21a1 expressions may possibly lead to enhanced testosterone, progesterone and 17-hydroxyprogesterone production in DVD-deficient brains. Reduction in 2-methoxyestradiol due to down-regulation of catechol-O-methyltransferase may contribute to develop preeclampsia which is a risk factor for ASD. Profiling these steroids in the AF is ongoing.

Poster 7 – Tuesday 6th December

USING 2-PHOTON IMAGING OF BRAIN CIRCUITRY TO EXPLORE THE MECHANISMS OF REPETATIVE TRANSCRANIAL MAGNETIC STIMULATION

Canty AJ[1], Bennett W[1], Tang AD[1,2], Hadrill C[1], Collins J[1], Rodger J[2], Garry MI[3], Hinder MR [3] and Summers JJ[3,4].

1. Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, TAS, Australia. 2. Experimental and Regenerative Neurosciences, School of Animal Biology, University of Western Australia Perth, WA, Australia. 3. Human Motor Control Lab, School of Medicine, University of Tasmania, Hobart, TAS, Australia. 4. Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK.

Modulation of cortical plasticity with repetitive transcranial magnetic stimulation (rTMS) has become a popular method of neuromodulation in both clinical and non-clinical populations. Typically used as a treatment for depression, repeated exposure to complex stimulation patterns such as intermittent theta burst stimulation (iTBS) have been shown to induce long lasting effects. Somewhat surprisingly, the biological mechanisms underpinning rTMS induced plasticity remain poorly understood, with few clues as to how such stimulation effects circuitry, and why there is such high variability in response between different patterns of stimulation and amongst individuals.
Rodent models offer the potential to investigate the structural and molecular mechanisms induced by stimulation. We combined a custom built rodent-specific TMS circular coil (8 mm outer diameter) and 2-photon imaging of Thy1-gfp mice through a cranial window to explore any synaptic changes induced by iTBS. We undertook repeated imaging of gfp labelled dendritic spines in the primary motor cortex (n=5 mice), both before and after a single iTBS session (120mT field strength, 600 pulses delivered over 190s over the motor cortex of awake, restrained mice) to look for any changes in circuitry. A single session of iTBS decreased dendritic spine density at 24hrs (-7% p=0.015) and 48 hrs (-9% p=0.001) post-stimulation which returned to baseline levels by 7 days post-stimulation (p=0.2). Such changes could correlate to altered motor output and transient changes in motor function.

Poster 8 – Monday 5th December

THE INTRINSIC APOPTOTIC PATHWAY- A DISTINGUISHING FEATURE AMONG SUDDEN INFANT DEATHS

N Ambrose[1,2], KA Waters[1,3] and R Machaalani, [1,3]

[1]Department of Medicine and The Bosch Institute, The University of Sydney, NSW 2006, Australia
[2] Department of Pathology, The University of Sydney, NSW 2006, Australia
[3]The Children's Hospital, Westmead, Sydney, NSW 2145, Australia

Over the past 2 decades, we identified upregulation of apoptotic markers in the brain in two cohorts of sudden infant death syndrome (SIDS) infants; a Canadian population (mid 1990's) and an Australian cohort (1997-2002). However, in those studies, the apoptotic pathway(s) involved were not studied. Of interest is the intrinsic pathway. This study aimed to (i) characterise a new infant dataset (2008-2012); (ii) investigate the immunohistochemical expression of three apoptotic markers; active caspase-3, active caspase-9 (intrinsic pathway marker), and TUNEL in nine nuclei of the rostral medulla of SIDS (n=27) and iii) compare results in SIDS cases with those where the cause of death was explained (eSUDI, n=10) as well as, for the first time cases listed as "undetermined or unascertained" (UD, n=23) by the pathologist and in two groups comprised of hypoxic-ischemic encephalopathy (HIE, n=5) and mechanical asphyxia (n=4). Similar to our previous reports, SIDS infants had higher caspase-3 expression in the hypoglossal and dorsal motor nucleus of the vagus when compared to eSUDI. Expression of active caspase-3 and caspase-9 were also greater in SIDS compared to UD cases, despite equivalence between the groups for demographics and risk factor profiles. TUNEL expression indicated that infant death occurred prior to DNA fragmentation in all groups. Levels of apoptosis were similar in SIDS and HIE groups, raising the possibility that SIDS infants experienced hypoxic events prior to death. This study is the first to identify a potential neuropathological difference in sub-groups of infants where the cause of death remains unknown.

Poster 9 – Tuesday 6th December

COPINE-6 REGULATES BASAL AND ACTIVITY-DEPENDENT TRAFFICKING OF AMPA RECEPTORS.

Jang SE[1], **Anggono V[1].**

1. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, 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. The levels of AMPARs on the postsynaptic membrane are tightly regulated by a number of intracellular scaffolding and signalling molecules. However, the precise mechanisms that regulate this process remain elusive. Copine-6 is an intracellular Ca^{2+} and phospholipid binding protein that has been recently identified to play a major role in structural and synaptic plasticity. We found that copine-6 forms a complex with AMPARs and regulates the expression of the GluA1 and GluA2 subunits under basal conditions, as well as during glycine-induced synaptic potentiation in cultured hippocampal neurons. Furthermore, we demonstrated that copine-6 translocates to syntaxin-13 positive endosomes, suggesting that copine-6 may play an important roles in regulating the endosomal trafficking of AMPARs.

Poster 10 – Monday 5th December

ACTIVITY-DEPENDENT REGULATION OF LONG NON-CODING RNA EXPRESSION IN PRIMARY CORTICAL NEURONS

Tan MC[1,2], Chau YQ[1,2], Cheung A[2], Widagdo J[1,2], **Anggono V[1,2].**

1. Clem Jones Centre for Ageing Dementia Research,
2. Queensland Brain Institute,
The University of Queensland, Brisbane, Queensland, Australia.

The ability of neurons to modulate the strength of their connectivity has long been postulated as a cellular correlate of learning and memory. It is well established that synaptic plasticity, such as long-term potentiation (LTP), requires the expression of plasticity-related genes and *de novo* production of new proteins. Recent advances in genomic sequencing technology have revealed that more than 90% of the genomes are actively transcribed, however, they are not translated into proteins. The long non-coding RNAs (lncRNAs) represent the major class of such transcripts and are highly expressed in the brain. However, their roles in synaptic plasticity, learning and memory remain elusive. By using a candidate screening approach, we profiled 90 well-annotated lncRNAs in primary cortical neurons at 10, 20 and 40 min following glycine stimulation by real-time quantitative polymerase chain reaction. Temporal analysis revealed dynamic expression of lncRNAs, many of which up- or down-regulated in the first 10 min post glycine application. Co-application of the NMDA receptor antagonist, D-APV, blocked the dynamic expression of these lncRNAs. These data suggest that the dynamics of lncRNA expression is tightly regulated by neuronal activity that mimics LTP and may play important roles in synaptic plasticity, learning and memory.

Poster 11 – Tuesday 6th December

INDUCED HAPLOINSUFFICIENCY OF KIT IMPAIRS CENTRAL NERVE DEVELOPMENT.

Aoki H, Kunisada T.

Department of Tissue and Organ Development, Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine, Gifu, Japan.

Kit receptor tyrosine kinase has been shown to regulate a wide range of biological functions in various cell lineages including pigment cells, hematopoietic cells, germ cells, neuronal cells of central nervous system (CNS). Here, we introduced a conditional loss of function mutation of *Kit* to induce *Kit* haploinsufficiency from a certain point of the developmental stage. Transmembrane region of *Kit* was flanked by loxP to excise the *Kit* floxed allele by Cre recombinase. To take advantage of CNS specific induction of Cre, $Kit^{floxed/+}$ mice were crossed with *Sox1-Cre* mice expressing Cre directed by the neural lineage specific promoter sequence. Expression of endogenous *Sox1* gene starts as early as E8.0 and *Kit* mRNA expression in their brain reduced into one half of the control in E10.5. The resultant $Kit^{floxed/+}$; *Sox1-Cre+* embryos showed significant reduction of the size of the forehead in E12.5, which generate a severe hypoplasia of CNS not only in their brain but also spine. These observations have not been previously reported in any *Kit* mutants, while functionally unknown expression of Kit has been found in neural stem cells and/or precursor cells *in vivo*. Our findings suggest that Kit expressed in developing CNS is functional signaling molecules necessary for proper brain development as the other cell lineages solely dependent for Kit in its certain developmental stage.

Poster 12 – Monday 5th December

THE TEMPORAL TUNING OF THE DROSOPHILA MOTION DETECTORS IS DETERMINED BY THE DYNAMICS OF THEIR INPUT ELEMENTS

Dr. Alexander Arenz[1], Michael Drews[1], Florian Richter[1], Georg Ammer[1], Prof. Dr. Alexander Borst[1]

[1]Max-Planck-Institute of Neurobiology

The direction of motion in the visual scene is not explicitly represented by the activity of single photoreceptors, but has to computed from the comparison of the activity of neighbouring photoreceptors across time. For this, classical algorithmic models of motion vision in vertebrates and invertebrates are based on the correlation of two input signals after differential temporal filtering. Recent experiments have challenged this view in *Drosophila* and found that elementary motion detectors rely on not only two, but at least three inputs. This is supported by connectomics data showing four cell types providing synaptic inputs onto the detectors. However, where the necessary temporal filters are implemented, which functional roles the different presynaptic neurons play and how they map onto the algorithmic

model is currently unclear. Here we comprehensively characterize the spatio-temporal response properties of all input elements onto the *Drosophila* motion detector neurons, T4 and T5 cells, by two-photon imaging. We find large differences in their temporal dynamics from fast band-pass to pure low-pass filters. Activation of the octopamine system accelerates the temporal filters, matching the tuning shift in T4/T5 neurons to higher velocities. Using computer simulations based on these filters under the two tuning conditions, we show that only very few of the possible input arrangements result in motion detectors with high direction selectivity. This way, we can suggest neural network implementations of the motion detectors in the fly.

Poster 13 – Tuesday 6th December

NEURODEGENERATIVE CHANGES BEGIN AS EARLY AS 1-MONTH POST-INJURY IN AN EXPERIMENTAL MODEL OF DIFFUSE MODERATE-SEVERE TRAUMATIC BRAIN INJURY

Arulsamy A.[1], Teng J.[1], Colton, H.[1], Corrigan F.[1], Collins-Praino L.E.[1]

Translational Neuropathology Lab, Discipline of Anatomy and Pathology, School of Medicine, University of Adelaide, Adelaide, Australia

Traumatic brain injury (TBI) is a known risk factor for the development of dementia. Despite this, the brain mechanisms that may account for this relationship are unknown. The current study investigated persistent structural changes and functional impairment post-TBI. Following diffuse moderate-severe TBI, Sprague-Dawley rats (n= 8-19/group) were tested on a battery for motor, cognitive and neuropsychiatric function at 7 days, 1 month or 3 months post-injury. Motor performance was significantly impaired at 7 days following TBI. Significant depressive-like symptoms, as measured by the forced swim test, emerged at 1-month post-TBI and persisted at the 3-month timepoint. Cognitive impairments, as measured by the Barnes maze, were seen at 3-months post-injury, although this did not reach statistical significance. While markers of structural integrity were significantly altered and oxidative stress was increased in the frontal lobe at 1-month post-injury, these neurodegenerative changes were no longer seen by 3-months post injury. Interestingly, while hippocampal tissue appeared normal, with the exception of elevated neuroinflammation, at 1-month post-injury, by 3-months post-injury, both synaptic and axonal integrity were reduced. Our data suggest that TBI causes motor impairment acutely post-TBI, with a later emergence of depressive-like symptoms at 1-month and cognitive impairments at 3-months post-TBI. These functional changes are associated with an emergence of neurodegenerative change first in the prefrontal cortex and later in the hippocampus. Taken together, this suggests that neurodegenerative changes may begin earlier than previously believed, and could predispose an individual for the later development of dementia.

Poster 14 – Monday 5th December

THE ANTERIOR CINGULATE CORTEX-PERIAQUEDUCTAL GRAY CONNECTOME CONSTITUTES THE AVERSIVE LIMBIC VOCALIZATION PATHWAY IN THE RAT

Arun M[1], Nagarajan V[2], Silburn PA[1], Subramanian HH[1]

[1]Queensland Brain Institute, Asia-Pacific Centre for Neuromodulation, The University of Queensland, Brisbane 4072, Australia.[2]Oxford University Medical School, John Radcliffe Hospital Oxford, United Kingdom OX3 9DZ

The midbrain periaqueductal gray (PAG) functions as the motor program generator for vocal behavior in mammals[1]. Limbic brain regions involved in emotional processing use the PAG to modulate vocalizations for emotional expression[2]. Of the very many limbic components, the anterior cingulate cortex (ACC) is involved in a range of emotional processing and vocal behaviour. Thus, important emotional commands to PAG may arise from ACC. However, it is not clear whether structural connections between the ACC and PAG exist. We investigated, in rats, ACC projections to the PAG via anterograde tracing and fixed brain tissue 16.4T diffusion weighted imaging (DWI) tractography. Furthermore, we investigated vocalizations that can be elicited from PAG areas innervated by ACC (if so), via chemical microstimulation. Anterograde tracing shows that ACC projects specifically to dorsal and lateral PAG. DWI tractography shows similar projections from the ACC to PAG and that density of the projections is comparable. Chemical stimulation of dorsal and lateral PAG produced (aversive) 22KHz ultrasonic vocalization calls, while ventrolateral PAG produced (positive) 50KHz ultrasonic calls. The existence of structural connection means the ACC contributes to the emotional processing of vocalizations evoked from the PAG. In particular, the ACC-PAG connectome may constitute the aversive limbic vocalization pathway in the rat.

1. Subramanian HH, Arun M, Silburn PA and Holstege G (2016). Motor organization of positive and negative emotional vocalization in the cat midbrain periaqueductal gray. *J.Comp. Neurol.* 524(8):1540-57.
1. Holstege G and Subramanian HH (2016). Two different motor systems generate human speech. *J.Comp. Neurol.* 524(8):1558-77.

Poster 15 – Tuesday 6th December

AN IN VITRO STUDY TO DETERMINE RELIABLE METHODS FOR EXAMINING THE EFFECT OF CANNABIDIOL IN EPILEPSY

Dr John Ashton[1], Shayma Ali, Assoc. Professor Steve Kerr

[1]University Of Otago

Background: One third of epilepsy patients are resistant to therapy. This is particularly true for childhood epilepsy, leading some parents to use an atypical cannabinoid drug, cannabidiol (CBD). This constitutes anecdotal, low quality evidence but recently completed placebo controlled trials however have shown more promising results, with CBD reducing seizures significantly compared to patients receiving placebo. How CBD reduces seizures is not known. Answering this question could help the development of other drugs for epilepsy.
Objective: To find experimental methods that can be used to study the effect of CBD on epileptic-like activity in the laboratory as a platform to study the mechanism of action of CBD
Method: Rat hippocampal slices were exposed to a variety of stimuli ex vivo to induce epileptiform activity, including electrical stimulation and the application of excitatory amino acids, and then responses to bath application of CBD tested.
Key findings: CBD was able to significantly reduce hyperexcitablity in slices exposed to kainic acid.
Conclusion: The kainic acid model of hippocampal slice hyperexcitablilty developed in this study may be used to investigate the mechanism of action of CBD.

Poster 16 – Monday 5th December

THE ROLE OF FTD/ALS ASSOCIATED PROTEIN, TDP-43, IN NEURITE AND SYNAPSE HEALTH AND FUNCTION

Rachel Atkinson[1], Jacqueline Leung[1], Carmen Fernandez-Martos[1], Julie Atkin[2], James Vickers[1], Anna King[1,]

[1]Wicking Dementia Research and Education Centre, University of Tasmania, Australia
[2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

A number of proteins have been identified that are pathologically or genetically associated with frontotemporal dementia and amyotrophic lateral sclerosis, including TDP-43. These diseases are characterized by significant neurite defects, including cytoskeletal pathology. The way in which TDP-43 is involved in the degeneration of neurons in disease is not yet well understood, however accumulating evidence shows its involvement in neurite outgrowth and maintenance by transporting cargo in neurites. Our initial studies have investigated the normal role of TDP-43 in development showing that protein levels are upregulated during neurite outgrowth and synapse formation. Using primary cortical neurons from homozygote TARDBP3c transgenic mice (n= 41 neurons) which overexpresses wild type (WT) human TDP-43 at 2.5x endogenous levels and C57/Bl6 littermates (n= 30 neurons), preliminary neurite tracing studies have demonstrated significant differences in the total length of all neurites (p=0.036), total number of all neurites (p=0.043) and number of primary neurites (p=0.033). We have also developed a model to study the role of pathogenic TDP-43 *in vivo* using the visual system as it allows non-invasive access to the CNS. Intraocular injection of WT TDP-43 AAV2 vectors using C57Bl/6 mice resulted in transduction of approximately 40% of retinal ganglion cells with no significant effect on vision at 1 month as tested with the optomotor response. Further studies will aim to determine the downstream effects of TDP-43 overexpression and mutant TDP-43. These studies will help us gain insight into the effect of disease-associated proteins and determine potential avenues for therapeutic intervention for axonal protection.

Poster 17 – Tuesday 6th December

LRP1 SUPPRESSES OLIGODENDROGENESIS IN THE ADULT MOUSE BRAIN

Loic Auderset[1], Dr CL Cullen[1], Prof Bruce Taylor[1], AssocProf Lisa C Foa[1], Dr Kaylene M Young[1]

[1]University of Tasmania

Oligodendrocyte progenitor cells (OPCs) are the immature cells that give rise to myelinating oligodendrocytes in the developing and mature central nervous system (CNS). The genes that regulate OPC behavior and oligodendrocyte maturation are poorly understood. However, recent microarray and RNA sequencing data has revealed that a number of genes are highly regulated across oligodendrocyte differentiation. One of these highly regulated genes is the low density lipoprotein receptor related protein 1 (*Lrp1*) which encodes a large transmembrane receptor known to interact with a variety of ligands. In the present study we have determined that LRP1 protein is highly expressed by OPCs and is rapidly downregulated at the early stages of oligodendrocyte differentiation. To determine the function of LRP1 in regulating OPC behavior, we used a cre-lox transgenic strategy to selectively ablate *Lrp1* from OPCs in early adulthood, and trace their fate over time. Tamoxifen was administered to P57 *Pdgfrα-CreER^{T2}::Rosa26-YFP* (control) and *Pdgfrα-CreER^{T2}::Rosa26-YFP::Lrp1$^{fl/fl}$* (gene deleted) mice. We found that OPCs lacking *Lrp1* produced significantly more new oligodendrocytes in the corpus callosum (P<0.001) and motor cortex (P<0.001) of the brain. This was accompanied by an increase in OPC density (P=0.02), suggesting that OPCs proliferate more rapidly in the brain of gene deleted than control mice. My data suggest that *Lrp1* is a potent negative regulator of oligodendrogenesis in the mature CNS.

Poster 18 – Monday 5th December

MODULATION OF NIBS-INDUCED PLASTICITY BY FLUOXETINE IN HEALTHY VOLUNTEERS

Doctor Duncan Austin[1], Doctor Lucia Li[2], Professor David Sharp[2], Professor John Rothwell[1]

[1]University College London, [2]Imperial College

Introduction

Non-invasive brain stimulation (NIBS) shows promise as a means of manipulating cortical plasticity, although limited by variability in individual responses. SSRIs are currently leading candidates for pharmacological agents for cortical 'priming' to improve variability, with evidence that they can modulate the effect of direct current stimulation (Nitsche, 2009) and enhance the effect of rehabilitative therapies (Chollet et al, 2012). We set out to compare the effect of fluoxetine on separate NIBS protocols, PAS25 and cTBS.

Methods
12 healthy participants (6M:5F, mean age 28 years, +/- 7yrs) completed 3 sessions of PAS25: baseline, placebo and fluoxetine 20mg. Each session used TMS to measure input-output curve, LICI (80, 150 & 200ms), SICI (2 & 3ms), ICF (10 & 15ms), and 1mV MEP measurements pre and 0, 15 and 30 mins post PAS25.

16 healthy participants (7M:9F, mean age 29.8 +/- 4yrs) received cTBS as an unmedicated baseline. Testing is now being repeated with fluoxetine + placebo.

Results
There was no effect of fluoxetine on SICI, ICF, LICI, PAS25 or input-output curve. In a 3-way repeat measures ANOVA, there was a significant effect of TIME ($p=.002$), with PAS25 producing a significant increase in MEP amplitude in each condition ($p<0.05$), but no TIME*CONDITION interaction ($p=.171$). In the baseline cTBS group, Post cTBS MEPs showed a significant inhibition ($p=.006$).

Conclusion
Single dose of fluoxetine did not modulate the effect of PAS25. Different NIBS protocols (TDCS, TBS) act on distinct cortical circuits and we are now exploring the effect of fluoxetine on cTBS.

Poster 19 – Tuesday 6th December

SEPARATE BANKS OF INFORMATION CHANNELS ENCODE SIZE AND ASPECT-RATIO.

Professor David Badcock[1], Dr Edwin Dickinson[1], Ms Sarah Morgan[1], Dr Matthew Tang[2]

[1]The University of Western Australia, [2]Queensland Brain Institute, The University of Queensland

OBJECTIVE: Size and aspect-ratio are ecologically important visual attributes. Relative size confers depth and aspect-ratio is a size-invariant cue to object identity. The aim is to determine the mechanisms of their analyses by the visual system. METHOD: In a series of 3 forced-choice psychophysical experiments (on a group of 4 experienced psychophysical observers) it is shown that adaptation causes perceptual repulsion in these properties and that they arise from separate perceptual estimates. FINDINGS: The results show significant adaptation effects in each observer and are consistent with the need for distinct banks of information channels tuned for different values of each property. CONCLUSIONS: Modelling of the data shows the necessary channels have log-Gaussian sensitivity profiles, with equal widths when expressed as ratios, labelled with their preferred magnitudes and distributed at exponentially increasing intervals. If an adapting stimulus reduces each channel's sensitivity in proportion to its activation then the displacement of the centroid of activity in the appropriate bank of channels due to a subsequently experienced test stimulus predicts the measured size or aspect-ratio aftereffect. The model also accounts for Weber's law, a fundamental property of human sensory performance; that the just noticeable difference in stimulus intensity approximates a constant fraction of that intensity.

Poster 20 – Monday 5th December

ENDOGENOUS OPIOIDS REGULATE MOMENT-TO-MOMENT NEURONAL COMMUNICATION AND EXCITABILITY

Elena Bagley[1], Bryony Winters[1], Gabrielle Gregoriou[1], Oliver Wells[1], Sarah Kissiwaa[1], Danashi Medagoda[1], Neil Burford[2], Andrew Alt[2], Hermes Sam[3], Sue Aicher[3]

[1]University Of Sydney, [2]Bristol Myer Squibb, [3]OHSU

Fear and emotional learning are modulated by endogenous opioids but the cellular basis for this is unknown. The intercalated cells (ITC) gate amygdala output and thus regulate the fear response. Here we find, using patch-clamp electrophysiology in brain slices from Sprague-Dawley rats, that endogenous opioids are released by minimal synaptic stimulation to act via two distinct mechanisms within the main ITC cluster. Endogenously released opioids significantly inhibit glutamate release through the delta-opioid receptor (DOR, 40.2 ± 4.8% inhibition, n=8, $p<0.01$ control vs ICI174864 , paired *t*-test), an effect potentiated by a DOR positive allosteric modulator, BMS-986187 (1μM). Post-synaptically, the opioids activate a potassium conductance through the mu-opioid receptor (MOR), suggesting for the first time that endogenously released opioids directly regulate neuronal excitability. Ultrastructural localization of endogenous ligands, using immuno-electron microscopy for met-enkephalin, support these functional findings. This study demonstrates a new role for endogenously released opioids as neuromodulators engaged by basal synaptic activity to regulate moment-to-moment neuronal communication and excitability. These distinct actions through MOR and DOR may underlie the opposing effect of these receptor systems on anxiety and fear.

Poster 21 – Tuesday 6th December

PHARMACOLOGICAL EVIDENCE THAT A FAILURE TO RECRUIT NMDA RECEPTORS CONTRIBUTES TO IMPAIRED FEAR EXTINCTION RETENTION IN ADOLESCENT RATS

Dr Kathryn Baker[1], Prof Rick Richardson[1]
[1]*School of Psychology, UNSW Australia*

Adolescents, both humans and rodents, exhibit a marked impairment in extinction of fear relative to younger and older groups which could be caused by a failure to efficiently recruit NMDA receptors (NMDARs) in adolescence. It is well-established that systemic administration of NMDAR antagonists (e.g., MK801) before extinction training impairs the retention of extinction in adult and juvenile rodents, but it is unknown whether this is also the case for adolescents. Therefore, in the present study we investigated the effect of pharmacologically manipulating the NMDAR on extinction retention in adolescent rats. When extinction retention is typically impaired (i.e., after one session of extinction training) adolescent male rats given D-cycloserine (a partial NMDAR agonist) showed enhanced extinction retention relative to saline-treated animals while animals given MK801 (an uncompetitive antagonist) did not exhibit any further impairment of extinction retention relative to the controls. In a further two experiments we demonstrated that when two sessions of extinction training separated by either 4 or 24 h intervals were given to adolescent rats, saline-treated animals exhibited good extinction retention and the animals given MK801 before the second session exhibited impaired extinction retention. These findings suggest that extinction in adolescence does not initially involve NMDARs and this is a likely mechanism that contributes to the impaired fear inhibition observed at this age. However, NMDARs appear to be recruited with extended extinction training or after administration of a partial agonist, both of which lead to effective extinction retention.

Poster 22 – Monday 5th December

NEUROPROTECTIVE EFFECTS OF APIGENIN AGAINST INFLAMMATION, NEURONAL EXCITABILITY AND APOPTOSIS IN AN INDUCED PLURIPOTENT STEM CELL MODEL OF ALZHEIMER'S DISEASE.

Miss Rachelle Balez[1], Dr. Martin Engel[1], Miss Sonia Sanz Muñoz[1], Mr Jeremy Stephen Lum[1], Dr. Lezanne Ooi[1]

[1]*Illawarra Health And Medical Research Institute*

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases, yet current therapeutic treatments are inadequate due to a complex disease pathogenesis. The plant polyphenol apigenin has been shown to have anti-inflammatory and neuroprotective properties in a number of cell and animal models; however a comprehensive assessment has not been performed in a human model of AD. Here we have used a human induced pluripotent stem cell (iPSC) model of familial and sporadic AD, in addition to non-AD controls, to assess the neuroprotective activity of apigenin. The iPSC-derived AD neurons demonstrated elevated Ab42/40 levels, a hyper-excitable calcium signalling phenotype, elevated levels of nitrite, increased cytotoxicity and apoptosis, reduced neurite length and increased susceptibility to inflammatory stress challenge. We identified that apigenin has potent anti-inflammatory properties with the ability to protect neurites and cell viability by promoting a global down-regulation of cytokine and nitric oxide release in inflammatory cells. In addition, we show that apigenin is able to protect iPSC-derived AD neurons via multiple means by reducing the frequency of spontaneous calcium signals and significantly reducing caspase-3/7 mediated apoptosis. These data demonstrate the broad neuroprotective action of apigenin against AD pathogenesis in a human disease model

Poster 23 – Tuesday 6th December

OVEREXPRESSION OF MUSK SUPPRESSES NEUROMUSCULAR TRANSMISSION IN THE MOUSE TIBIALIS ANTERIOR MUSCLE

Miss Joanne Ban[1]

[1]*Physiology, University Of Sydney*

Muscle Specific (tyrosine) Kinase (MuSK) is expressed in the postsynaptic membrane of the neuromuscular junction (NMJ) where it stabilizes the co-localized acetylcholine receptors. The influence of MuSK in synaptic physiology remains uncertain. To investigate this we used adeno-associated viral vector (AAV) to over-express MuSK-GFP. The right tibialis anterior (TA) muscle of 8-week old mice was injected with AAV-MuSK-GFP. The contralateral muscle was injected with empty AAV vector. Three weeks later, MuSK-GFP was strongly expressed in the postsynaptic membrane of the NMJ. Mice were anaesthetized with isoflurane and maximum tetanic force was recorded in response to stimulation of the sciatic nerve or via direct muscle stimulation. Muscles injected with empty vector produced steady tetanic force for the first 400msec of nerve stimulation. Longer stimulus trains (800msec and 1600msec) produced modest decay in nerve-evoked force. In contrast, muscles injected with AAV-MuSK-GFP showed significant decay in nerve-evoked force during a 400msec tetanus (14.5 ± 1.81, P<0.0001, n=14). Longer trains of nerve stimuli produced significantly greater loss tetanic force 34.4 ± 2.9% and 54.5 ± 3.6%, compared to 12.9 ± 1.7% and 28.2 ± 2.68% for empty vector control muscles (n=14). Following the end of the 400ms tetanus, muscles expressing MuSK-GFP recovered 50% of their original peak force after a 3.6s delay. These results suggest that MUSK kinase system may negatively regulate neuromuscular transmission through an, as yet unidentified, mechanism.

Poster 24 – Monday 5th December

EPITHELIAL NERVE FIBRES OF THE VAGINA OF NULLIPAROUS AND MULTIPAROUS MICE

Dr Christine Barry[1], Ms Esther Ji[1,2], Ms Harman Sharma[1,2], Mrs Patricia Vilimas[1,2], Professor Rainer Haberberger[1,2]

[1]Anatomy and Histology, Flinders University, [2]Centre for Neuroscience, Flinders University

Vulvodynia is a chronic genital pain disorder affecting millions of women and girls worldwide with direct medical costs exceeding $20 billion p.a. (US figures). It is under-researched and associated with hyperinnervation of the vagina. Normal vaginal innervation is poorly documented and it is unknown which populations of fibres contribute to hyperinnervation in vulvodynia.
This study aimed to characterise epithelial innervation of the murine vagina using triple labelling immunohistochemistry and high magnification confocal image analysis. Using the pan-neuronal marker PGP9.5 we identified fibre subpopulations immunoreactive (IR) for vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), substance P (SP) and neuropeptide tyrosine (NPY). As risk factors for vulvodynia include vaginal delivery, we compared innervation in nulliparous (NP) and multiparous (MP) mice (n = 5 per group).
Epithelial innervation was equally dense in the distal vagina of NP and MP mice and present sparsely in the proximal vagina of some mice (3 NP, 1 MP). A third of epithelial fibres in the distal vagina were non-peptidergic, similar proportions contained IR for CGRP with and without SP or VIP, 10% were VIP+CGRP-, 5% SP+CGRP- and none NPY+. All epithelial fibres of the proximal vagina were peptidergic. Compared to NP mice, MP mice showed similar epithelial innervation density but higher proportions CGRP+SP-VIP- and nonpeptidergic fibres in the distal vagina ($p < 0.0001$).
These studies show the murine vagina contains multiple populations of epithelial nerve fibres that could contribute to nociception. Results show changes in neurochemical characteristics in MP mice with proliferation of presumptive nociceptors.

Poster 25 – Tuesday 6th December

WHO ARE WE? WHAT ARE WE? WHAT DO WE WANT TO BECOME?

David Basser[1]

[1]School of Philosophy, UTas

Within and beyond therapeutic uses, current and emerging biotechnologies, including neurotechnologies, provide the opportunity to make quantum changes to our physical and emotional selves, and our environment. While we stand at this major inflection moment on the journey of humankind, the choices we make will determine the character of the humanscape that evolves following this irreversible dimensional shift. As such, it is our individual and collective responsibilities to current and future generations to consciously consider, contemplate and answer the enduring and relevant philosophical questions: Who are we? What are we? What do we want to become? A glimpse of the emerging possibilities and sequelae may be inferred through the window of real-time experiences associated with current therapeutic applications. Deep brain implants used for the treatment of Parkinson's disease provides one such window.

Poster 26 – Monday 5th December

EVALUATION OF BRAIN LESIONS IN AN OVINE MODEL OF ACUTE NEURONOPATHIC GAUCHER DISEASE: CORRELATION WITH HUMAN DISEASE

Leanne Winner[1], **Helen Beard[1]**, Dr Nicholas Smith[2], Proffessor John Hopwood[1], Dr Lisa Karageorgos[1], Dr Kim Hemsley[1]

[1]South Australian Health and Medical Research Institute (SAHMRI), [2]Department of Neurology, Women's and Children's Hospital

Acute neuronopathic (type II) Gaucher disease (GD) is a devastating neurological disorder resulting from mutations in the glucocerebrosidase gene, with subsequent accumulation of glucosylceramide and glucosylsphingosine. Rapidly progressive neurodegeneration begins in infancy and progresses to death, typically before two years of age. Our objective was to examine disease lesions in the brain of GD lambs and relate the findings to those in human post-mortem tissue to determine the validity of the lamb as a model of human GD. Newborn GD (n=5) or unaffected lamb (n=9) brain samples were immersion-fixed in 4% paraformaldehyde, processed into paraffin, with sections cut/stained immuno-/histochemically. Astro-/microgliosis (GFAP/isolectin B4, respectively), endo/lysosomal system expansion (LIMP-2; cathepsin D) and intra-neuronal lesions (p-Tau; ubiquitin) were evaluated quantitatively using thresholding/lesion counting, or qualitatively (lesions present: yes/no). The examiners remained blind to the genotype of the sample throughout. We observed significant increases in LIMP-2 staining in GD lamb cingulate gyrus ($p<0.01$), however cathepsin D levels remained either unchanged or reduced ($p<0.05$), depending on brain region. The appearance of activated, amoeboid microglia was noted throughout the GD lamb brain but no significant astroglial response was observed in any brain region assessed. p-Tau-positive inclusions were present in subcortical white matter (WM) and the optic tract, and ubiquitin-positive inclusions and/or cytoplasmic puncta were present in many brain regions including the thalamus and subcortical WM of GD lamb brains. Our findings indicate differences in the manifestation of disease in human versus lamb type II GD brain.

Poster 27 – Tuesday 6th December

TAU KNOCKOUT MICE AS A MODEL FOR PRE-MOTOR PARKINSON'S DISEASE-RELATED HYPOSMIA

Miss Leah C Beauchamp[1], Dr Laura J Vella[2,3], Mr Jacky Chan[2], Dr Laura H Jacobson[1,2], Associate Professor Kevin J Barnham[1,2,3]

[1]The Department of Pharmacology and Therapeutics, The University of Melbourne, [2]The Florey Institute of Neuroscience and Mental Health, [3]Bio21 Insitute of Molecular Science and Biochemistry, The University of Melbourne

The etiology and pathogenesis of Parkinson's disease (PD) remain enigmatic. Recent evidence implicates the protein tau in the development of idiopathic PD, although the role of tau in PD is not well understood. Clinical onset of PD is analogous with the presentation of motor symptoms, which are the result of midbrain substantia nigra neurodegeneration. In order to develop treatments that prevent advancement of PD, there is a need for greater understanding of the early pathobiology of the disease. Characterisation of early, non-motor symptoms of PD, such as hyposmia, may provide insights that open avenues for novel drug target discovery and future drug therapies.
Tau knockout ($^{-/-}$) mice have been proposed as an age-dependent model of PD as they show motor and cognitive impairments and pathobiological PD-like features at 12 months of age. We therefore investigated tau$^{-/-}$ mice for premotor PD-like changes in the olfactory system. Hyposmia was evident in tau$^{-/-}$ mice at 7 mo of age, in the absence of motor impairments, with a possible onset at 2 months of age. Tyrosine hydroxylase expression in the olfactory bulb was elevated in tau$^{-/-}$ mice, similar to that of post-mortem PD brain. By 7 mo of age there were indications of impaired autophagy in the olfactory bulbs of tau$^{-/-}$ mice, concurrent with an accumulation of α-synuclein. We have discovered that tau$^{-/-}$ have pre-motor hyposmia that is analogous with PD. These data support the tau$^{-/-}$ mouse as a discovery tool in PD research, and implicates a pathological role for tau in early PD.

Poster 28 – Monday 5th December

HIGH FAT DIET IN ACTION CONTROL: A NEW ROLE FOR MICROGLIA

Becchi S.[1], Kendig M.[2], Hood J.[2], Balleine BW.[1], Corbit L.[2]

[1] University of New South Wales, Sydney, NSW, Australia.
[2] University of Sydney, Sydney, NSW, Australia.

It is well established that obesity is associated with peripheral inflammation and with changes in microglia response in various areas of the brain. Our aim is to understand the effects of an obesogenic diet on microglia response in key areas for goal-directed and habitual behavioural control and therefore its effect on synaptic plasticity and learning.
The role of microglia in the control of goal-directed and habitual behaviour in rats was investigated by giving 5 weeks of access to chow with or without sweetened condensed milk (SCM) before instrumental training, and then measuring the impairment of goal-directed performance using the outcome devaluation task. Control rats reduced responding following devaluation of the earned outcome whereas rats with previous access to SCM responded similarly under the devalued and nondevalued conditions, indicating loss of goal-directed control of responding. Using analyses of 3-D morphological reconstructions of microglia cells we found an increase in microglia number and size, particularly in the dorsomedial striatum (DMS), important for goal-directed learning following chronic access to SCM. Pharmacological blockade of microglial activation with minocycline restored goal-directed control following chronic SCM.
These results indicate that an obesogenic diet can promote microglial activation in decision-making circuits undermining the normal function of the goal-directed DMS system allowing early habitual control mediated by the dorsolateral striatum (DLS) circuit. These results have important implications for understanding failures of behavioural control and strategies for improving behavioural flexibility.

Poster 29 – Tuesday 6th December

SIGNIFICANT IMPROVEMENT OF OLFACTORY REGENERATION IN MICE FOLLOWING TREATMENT WITH VEGF AND PDGF GROWTH

Beecher K[1], Hafner L M[1], Ekberg J[2], St John J[3], and Chehrehasa F[1&3]

1. School of Biomedical Sciences, Queensland University of Technology, 2 George St, Brisbane City, QLD 4000, Queensland, Australia
2) Faculty of Health and Medical Science, Bond University, 14 University Drive, Robina 4226, Queensland, Australia
3) Clem Jones Centre for Neurobiology and Stem Cell Research, Eskitis Institute for Drug Discovery, Griffith University, 170 Kessels Rd, Nathan 4111, Brisbane, Queensland, Australia

Anosmia due to large-scale olfactory nerve damage from skull base surgery is an important problem for human health that significantly influences physical wellbeing, quality of life, and life-expectancy in those affected. Therefore, there is a need to find a treatment for anosmia. The VEGF and PDGF growth factor treatments have improved regeneration of the CNS after injury. Therefore, we have tested the roles of the combined growth factors in improvement of olfactory regeneration in mice. The degeneration of the murine olfactory neurons was induced by unilateral surgical removal of the olfactory bulb. The animals were randomly divided into two groups: the treatment animals group received 0.5 µg of the combined growth factors and the control animals received PBS. Combined growth factor and PBS was intranasally administered over three days, and tissues were harvested from the two groups at different time points post-surgery and histological techniques were used for analysis.
We have found that olfactory regenerating axons projected significantly deeper to the brain in the treatment group compared to the control group at day 10 and 14 ($p < 0.05$) and the thickness of the olfactory epithelium was significantly increased after 10 days post- surgery in the treatment group ($p < 0.05$).
The results indicate that the combined delivery of VEGF and PDGF growth factor treatments has improved regeneration of olfactory neurons in this murine model and has the potential to restore the sense of smell in humans with anosmia.

Poster 30 – Monday 5th December

EFFECT OF TRANSPORT AND ACTIVITY OF DETEMIR INSULIN IN THE CENTRAL NERVOUS SYSTEM ON ENERGY BALANCE

Dr Denovan Begg[1]

[1]UNSW 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. Transport of insulin into the brain is altered by numerous factors including diet induced obesity. We have previously reported 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 C57BL/6J mice. Acute 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. When mice were maintained on a high fat diet the acute effects of detemir insulin on both energy balance and phosphorylated Akt were inhibited. These data suggest 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 central nervous system.

Poster 31 – Tuesday 6th December

CHANGES IN DENDRITIC STRUCTURE AND SPINE DENSITY PRECEDE NEURON LOSS IN SUB-CORTICAL, BRAINSTEM AND SPINAL CORD AREAS IN THE HSOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Dr Matthew J Fogarty[1], Dr Erica W H Mu[1], Dr Peter G Noakes[1,2], Dr Nickolas Lavidis[1], **Dr Mark C Bellingham[1]**

[1]School of Biomedical Sciences, The University Of Queensland, [2]Queensland Brain Institute, The University of Queensland

Amyotrophic lateral sclerosis (ALS) is a characterised by the death of upper and lower motor neurons (MNs) with progressive muscle weakness. This incurable disease is clinically heterogeneous and its aetiology remains unknown. Electrophysiological studies have found increased excitability in both upper and lower MNs prior to neuron loss and symptom onset in human or rodent studies. Increased neuronal excitability has been correlated with structural changes in neuronal dendritic arbors and spines for decades. Here, using a modified Golgi-Cox staining method, we report the first longitudinal study examining the dendrites of CA1 pyramidal neurons of the hippocampus, medium spiny neurons of the striatum and resistant (trochlear, IV) and susceptible (hypoglossal, XII; lumbar) MNs from the brainstem and lumbar spinal cord of transgenic mice over-expressing the human $SOD1^{G93A}$ (SOD1) mutation, compared to wild-type (WT) controls, at postnatal (P) days 8-15, 28-35, 65-75 and 120. We describe structural changes commencing at pre-symptomatic ages (P8-15 or P28-35) in spinal cord lumbar MNs in SOD1 compared to WT mice. Spine loss without concurrent dendritic changes was present in striatal neurons from disease-onset (P65-75). Spine density increases were present at all ages studied in SOD1 XII MNs. Spine density increased in neonatal lumbar MNs, before decreasing to control levels by P28-35 and were decreased compared to WT controls by P120. Vacuolisation of SOD1 XII MNs and lumbar MNs occurred from the same time-points. Trochlear MNs did not exhibit significant vacuolisation or dendritic changes at any time point studied. Our results suggest that these changes in dendritic structure and in dendritic spine density correlate with the neuromotor phenotype in ALS, and with the varied cognitive and extra-motor symptoms commonly seen in patients. Further, we show that changes in dendritic arbors and spines differ for susceptible cranial (XII) MNs and spinal cord (lumbar) MNs, but are conspicuously absent in cranial (IV) MNs resistant to loss in ALS. Determining if these phenomena are compensatory or maladaptive may help explain differential susceptibility of MNs to degeneration

Poster 32 – Monday 5th December

ASTROCYTIC MODULATION OF NEURONAL NETWORK OSCILLATIONS

Alba Bellot-Saez[1,2], Greg Cohen[2], John Morley[1,2], Yossi Buskila[1,2]
[1]School of Medicine, Western Sydney University, [2]Biomedical Engineering and Neuroscience group, The MARCS Institute, Western Sydney University

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 gears the transition between different oscillatory frequencies is still unknown.
In this study, we have explored the potential involvement of astrocytic K^+ clearance process in the modulation of neural oscillations at the network level. Our results indicate that local increase in extracellular K^+ concentration leads to local network hyperexcitability, as evidenced by enhanced firing frequencies and membrane potential depolarization. Hyperexcitable neuronal tissue displayed an increase in oscillation frequencies and amplitude across a wide spectrum. Reduced astrocytic K^+ buffering capabilities through bath application of Barium at low concentration, as well as selective blockade of astrocytic connectivity, resulted in increased network excitability, as indicated by decreased inter-spike intervals and longer excitability durations.
Since astrocytes are crucial for maintaining brain homeostasis by means of K^+ spatial buffering through their gap junction-mediated connections, our study suggests that modulation of their inherent capabilities to clear K^+ from the extracellular milieu is a potential target to impact neural oscillations, and thereby tuning the transition between brain waves.

Poster 33 – Tuesday 6th December

MODELLING EXCITOTOXIC INDUCED AXON DEGENERATION IN THE VISUAL SYSTEM

Mr James Bender[1], Dr Jacqueline Leung[1], Dr Carmen Fernandez-Martos[1], Dr Nuri Guven[2], Professor James Vickers[1], Dr Anna King[1]

[1]Wicking Dementia Research And Education Centre, [2]School of Medicine, Faculty of Health Science, University of Tasmania

Axon degeneration is a key pathological process in neurodegenerative diseases and may result in neuronal disconnection. However, little is known about the mechanisms of axon degeneration that occur under the pathogenic mechanisms of disease. Excitotoxicity is a pathological process known to occur in a variety of these neurodegenerative diseases and has been shown to be capable of inducing axonal degeneration. Cell cultures studies indicated that excitotoxin induced axon degeneration involves axonal caspase activation and disruption to microtubules, however, mechanisms have not been confirmed *in vivo*. This study aimed to develop and characterize a CNS model of excitotoxic axon degeneration. The visual system is a part of the CNS that is amenable to analysis of axon degeneration. Thus kainic acid was injected into the vitreous humour of the eye in mice to expose the retinal ganglion cells to the excitotoxin. Downstream effects were observed using visual function testing, histology and immunohistochemistry.
Intravitreal injection of kainic acid resulted in a significant loss of visual acuity by 1-day post-treatment ($p=0.039$). Histologically there was a significantly ($p=0.007$) increased immunoreactivity of the astrocyte marker GFAP, indicating an astroglial response. Additionally, analysis of retinal ganglion cell axons suggested increased expression of the intermediate filament protein alpha-internexin as well prominent changes to neurofilament proteins including formation of neurofilamentous swellings within the axon. These data increase our understanding of axon degeneration in neurological disease and injury and provide an *in vivo* model to assess future therapeutic interventions to protect axons, such as the microtubule stabilising agent, epothilone D.

Poster 34 – Monday 5th December

DEVELOPING A NEW ZEALAND SHEEP MODEL OF HEMI-PARKINSON'S DISEASE.

Dr Dave Bergin[1,2,3], Mr Jason Gray[1,2,3], Mr Dave Matthews[4], Ms Fiona Park[4], Dr Lisa Smith[1,2,3], Dr Chris Perk[1,2,3], Professor Brian Hyland[2,3,5], Associate Professor John Reynolds[1,2,3]

[1]Department of Anatomy, University Of Otago, [2]Brain Health Research Centre, University of Otago, [3]Brain Research New Zealand, University of Otago, [4]Hercus Taieri Resource Unit, University of Otago, [5]Department of Physiology, University of Otago

Parkinson's disease is a movement disorder caused by the death of dopamine producing neurons in the brain. Dopaminergic toxins 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are used in rats and primates respectively, to model the disease process and to investigate potential therapies. Thus far, the only neurotoxin model employing sheep used MPTP. MPTP is administered systemically and poses significant health risks to both animal and experimenter.
The aim of the present study was to establish a safer unilateral neurotoxic lesion model in sheep using a local injection of 6-OHDA into the left substantia nigra. To quantify lesion extent, the apomorphine challenge as used in rats was employed, wherein systemic administration of the dopamine agonist apomorphine drives circling behaviour due to the dopamine system imbalance between lesioned and unlesioned hemispheres.
We found (i) the dose of apomorphine determined the direction of turning, (ii) the amount of toxin decreased the apomorphine dose threshold for switching contra to ipsiversive turning, (iii) apomorphine made unlesioned sheep hyperactive without obvious turning behaviour and (iv) isolated limb dyskinesias could be induced in some animals by repeated high doses of apomorphine alone.
The present study illustrates the use of stereotaxic surgery in sheep as a large animal model of a late stage neurodegenerative disease, specifically Parkinson's disease, thereby removing the health risks associated with MPTP. This study also highlights the possibility for non-primate, non-genetic, non-systemic but locally engineered large animal experimental models using sheep.

Supported by the Holdsworth Charitable Trust

Poster 35 – Tuesday 6th December

INTRACELLULAR SIGNALLING PATHWAYS AND DIRECTIONALITY OF ENTERIC NEURAL CREST CELL MIGRATION

Annette Bergner[1], Huyhn Nguyen[1], Professor Heather Young[1]

[1]Department Of Anatomy and Neuroscience

Most of the enteric nervous system arises from neural crest-derived cells that emigrate from the caudal hindbrain and then migrate into and along the developing gut. As enteric neural crest-derived cells (ENCC) are migrating along the gut, some cells must migrate caudally into un-colonized regions, while other cells must remain behind in each region to ensure there are enteric neurons along the entire gut. It had been assumed that each gut region was populated by ENCCs that had stopped migrating. However, we recently showed that each region is populated by ENCCs that migrate non-directionally rather than caudally. The mechanisms that control ENCC directionality are unknown. In this study we performed live cell imaging and examined the effects of perturbing JNK, MEK and PKA on the speed and directionality of individual ENCCs. A minimum of 50 control and drug-treated cells were examined for each drug. The JNK inhibitor, SP600125, significantly decreased ENCC speed, but had no effect on ENCC directionality. The MEK inhibitor, PD184352, had no significant effect on ENCC speed or directionality. SP-8-Br-cAMP, an activator of cAMP-dependent kinases, had no effect on speed but significantly increased the caudal directionality of ENCCs. Forskolin, an adenylyl cyclase activator, decreased ENCC speed and increased caudal

directionality of ENCCs. However, Rp-cAMPS, a PKA inhibitor, had no effect on ENCC speed or directionality. We conclude that different levels of cAMP signalling may contribute to the variable directionality of individual ENCCs.

Poster 36 – Monday 5th December

EARLY DEVELOPMENTAL RETINAL GANGLION CELL DEATH AND SHORT-RANGE AXONAL TARGETING ERRORS

Mr Jamie Beros[1,2], A/Prof Jennifer Rodger[1], E/Prof Alan Harvey[2]

[1]School of Animal Biology, The University of Western Australia, [2]School of Anatomy, Physiology and Human Biology, The University of Western Australia

During development of the visual system, retinal ganglion cells (RGCs) in the retina undergo extensive apoptotic death. In the mouse, approximately 50% of the RGCs present at birth (postnatal day 0; P0) die by P5, coinciding with axonal innervation of a major target, the superior colliculus (SC). This study aimed to determine if RGCs making short-range axonal targeting errors in the contralateral SC are more likely than RGCs making accurate connections to be eliminated during the peak period of RGC death (P1-P5). The retrograde nucleophilic dye Hoechst 33342 (H; 2.3nl) was injected into the left SC of anaesthetised neonatal C57Bl/6J mice at P1 or P4 (n=5 and 9), and retinas wholemounted 12 hours later. Retrogradely labelled healthy and dying RGCs were identified by morphological criteria. Percentage of RGC death was analysed in relation to distance from the area of highest density RGC labelling, presumed to represent a topographically accurate population. The P1 cohort had a greater overall amount of RGC death compared to P4 cohorts ($p<0.05$), however, there were no differences in RGC death varying with distance from the centre of the densely labelled area. We confirmed that developmental RGC death was greater at P1 than P4. However, the lack of association between short-range axonal targeting errors and apoptotic profiles suggests that death may occur independently of topographic accuracy. Future studies will investigate whether other factors such as RGC birthdate may contribute to the biological triggers for neuronal death during development.

Poster 37 – Tuesday 6th December

CORRECTION OF AUTOSOMAL, LYSOSOMAL, AND SYNAPTIC CHANGES PRESENT IN OVINE NEURONAL CULURES OF NEURODEGENERATIVE BATTEN DISEASE

BEST HL[1,3], NEVERMAN NJ [1,3], WICKY HE [1,3], MITCHELL NL [2,3], PALMER DN [2,3] AND HUGHES SM[1,3].

[1]Department of Biochemistry, Brain Health Research Centre, University of Otago, Dunedin.
[2]Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand.
[3]Batten Animal Disease Network, BARN.

Batten disease is a group of inherited childhood neurodegenerative lysosomal storage disorders characterised by progressive visual, motor, and mental decline. Currently, only palliative therapies are available and the disease mechanisms are unknown. $CLN5^{-/-}$ and $CLN6^{-/-}$ disease forms have been identified in two New Zealand sheep flocks. Neural cells, isolated from these sheep, are maintained in dissociated cultures, providing an invaluable resource for elucidation of disease pathways and therapeutic testing. The aims of this study were to characterise early cellular changes *in vitro*, and to use these changes as biomarkers for therapeutic testing. We have previously identified synaptic and autosomal-lysosomal pathway (APL) changes in $CLN6^{-/-}$ neural cultures. This data was supported by transmission-electron microscopy showing increased numbers of autolysosomes containing storage material, indicative of ALP dysfunction. Utilising the same assays, we found that $CLN5^{-/-}$ cultures had a 23% decrease in autophagic flux ($p = 0.0016$), a 44% decrease in synaptic activity-dependent bulk endocytosis of fluorescent dextran ($p = 0.0039$), and a 34% decrease in acidic vesicles ($p = 0.0006$), when compared to healthy controls. In response to the changes in ALP activity, we tested the therapeutic potential of two fibrate drugs, fenofibrate and gemfibrozil; both known enhancers of autophagy and lysosomal gene expression. Gemfibrozil significantly increased autophagic flux (53% increase, $p < 0.01$), acidic vesicles (95% increase, $p < 0.01$) and relative gene expression of *LAMP1* ($p = 0.0129$). This data reveals common defects between $CLN5^{-/-}$ and $CLN6^{-/-}$ Batten disease and warrants further investigation into the therapeutic potential of fibrate drugs.

Poster 38 – Monday 5th December

BICEPS STIMULATION TO IMPROVE RESPIRATION AFTER CERVICAL SPINAL CORD INJURY

T. Bezdudnaya, V. Marchenko, M. Melconian, K. M. Hormigo, M. Randelman, L. Zholudeva, M.A. Lane

Drexel University College of Medicine, Philadelphia, USA

Cervical spinal cord injury (SCI) typically disrupts phrenic motor circuitry which mediates diaphragm function, necessitating assisted ventilation, and placing individuals at risk of mortality. Finding a treatment that helps to eliminate ventilator dependence and restore impaired diaphragm function will improve quality of life and reduce the risk of mortality in these patients. We are focused on developing a less-invasive therapeutic strategy that employs upper extremity muscle electrical stimulation to activate injured phrenic motor output and promote respiratory recovery. The biceps brachii muscle was selected as its motor circuitry is located in the same cervical levels as the phrenic motoneurons (C3-C5).
Adult Sprague-Dawley rats were used for all experiments. Animas received a lateral hemisection (Hx) at C2 level. C2Hx interrupts inputs from brainstem to ipsilateral phrenic motoneurons and results in hemidiaphragm paralysis. Decerebrate, unanesthetized and artificially ventilated rats were used for terminal electrophysiology to examine the effect of biceps stimulation on the phrenic activity immediately

following SCI. Our experiments demonstrated that closed-loop stimulation of the biceps muscle, triggered by the animal's own inspiratory activity, increases phrenic motor output ipsilateral to injury. Anatomical studies with dual, transynaptic, retrograde tracing from the diaphragm and biceps using pseudorabies virus revealed double-labeled pre-motor interneurons. These interneurons anatomically integrate the two circuits at the spinal level and may provide a basis for enhancing of respiratory recovery. Building upon this promising result,ongoing work is now assessing whether daily closed-loop biceps brachii stimulation can be employed to promote respiratory plasticity in chronically injured animals.
Supported by Conquer Paralysis Now (CPN) and the Edward Jekkal Muscular Dystrophy Association Fellowship (Drexel).

Poster 39 – Tuesday 6th December

INVESTIGATING THE PROPAGATION OF ALPHA-SYNUCLEIN PATHOLOGY IN A MOUSE MODEL OF PARKINSON'S DISEASE

Gregor Bieri[1,2], Michel Brahic[2], Aaron Gitler[2], Ronald Melki[3]

[1]Stanford University, Neurosciences PhD Program, [2]Department of Genetics, Stanford University School of Medicine, [3]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS

Mounting evidence suggests that aggregation-prone proteins, associated with a variety of neurodegenerative diseases, propagate and spread in a prion-like way through interconnected neural networks. For alpha-synuclein (a-syn), a key component of the Lewy body pathology in Parkinson's disease (PD), the mechanism whereby it is transmitted through neuronal pathways is unknown. To study the propagation of a-syn pathology, we injected recombinant fibrillar a-syn into dorsal striatum of adult mice. Functional, structural and pathological changes were assessed up to six-months post-injection using behavioral assays and histological markers of aggregation, degeneration and gliosis. Mice injected with a-syn displayed altered anxiety-related behavior at late time points, but no differences in motor skills. We observed a-syn inclusion away from the injection site in both cortical hemispheres, amygdala and unilaterally in the substantia nigra (SNpc). Furthermore, we detected a decrease in TH-positive processes and increased microglial activation in areas displaying a-syn pathology. To gain mechanistic insights into genetic interactions leading to PD, we focused on prevalent mutations in the LRRK2 gene associated with familial PD (fPD). We measured aggregation, spread and survival in mutant fPD human iPS-derived neurons, and primary mouse neurons, and observed altered transport kinetics and enhanced a-syn aggregation in mutant neurons. Additionally, we injected a-syn fibrils into the brains of LRRK2 mutant mice and detect impaired behavioral performance, increased a-syn aggregation in SNpc neurons and altered microglial activation. Collectively, our data establish a robust a-syn spreading model that can be used to investigate disease-modifying genetic interactions and potential therapeutic interventions for PD.

Poster 40 – Monday 5th December

IMMUNE CELL INFILTRATION, ACTIVATION, AND INFLAMMATION PLAYING A ROLE IN AGEING OF THE MOUSE INNER EAR VESTIBULAR (BALANCE) SYSTEM

Mr Mark Bigland, Professor Alan Brichta, Dr Doug Smith

[1]School of Biomedical Sciences & Pharmacy University of Newcastle, [2]Priority Research Centre for Brain and Mental Health Research, [3]Hunter Medical Research Institute Australia

Background: Maintenance of balance declines with age, often leading to falls requiring hospitalisation. A contributing factor to vestibular decline is thought to be impaired vestibular function, however, it is unclear whether age-related vestibular dysfunction is due to peripheral and/or central components. Vestibular hair cells detect head motion and deterioration of hair cell function would be detrimental to overall vestibular function
Objective: Our study sought to determine whether peripheral vestibular organ function is compromised with ageing by characterising molecular changes that might contribute to age-related balance disorders.
Methods: We performed microarrays on RNA samples from young (3.5 months), middle (14 months), and old age (>28 months) mouse peripheral vestibular organs. Array data was used to determine differential gene expression with age, on Affymetrix GeneArrays (ANOVA $P < 0.01$, False Discovery Rate < 0.25, Fold Change > 1.5). Differentially expressed gene lists were subjected to DAVID for identification of enriched biological pathways.
Results: Ageing significantly increased the expression of genes associated with immune response pathways: e.g. Asthma, Primary immunodeficiency, Immune network for IgA production, Autoimmune thyroid disease, B cell receptor signalling, Systemic lupus erythematosus, Natural Killer cell mediated cytotoxicity, and Calcium signalling. Downregulated genes were associated with: sensory transduction, ion homeostasis, and inner-ear development.
Conclusions: These data suggest immune cell infiltration, activation, and inflammation, is instrumental in age-related peripheral balance disorders. Further evidence suggests these changes may be triggered by breakdown of the blood labyrinth barrier as seen in other tissues with ageing. Further research in this area is required.

Poster 41 – Tuesday 6th December

IDENTIFICATION OF A UNIQUE GABAA RECEPTOR ALPHA SUBUNIT SPLICE VARIANT

Mr Sam Pelly[1], Ms Stephanie Miller[1], Dr Viska Kalanjati[1], Prof Paul Colditz[1], **Dr Tracey Bjorkman[1]**

[1]*Perinatal Research Centre, UQCCR, The University Of Queensland*

$GABA_A$ receptors provide the majority of inhibitory responses in the adult central nervous system but during development provide excitatory input. The $GABA_A$ receptor is composed of 5 subunit proteins which determine receptor function and pharmacology; expression of these subunits differs between brain regions and varies significantly with brain development. The $GABA_A$ receptor □$_3$ subunit isoform is the most prominent □ subunit during brain development.
Western blot studies in our laboratory routinely identify a smaller but specific protein that reacts to an antibody directed towards the $GABA_A$ subunit α_3 protein in the neonatal pig brain. The aim of this study was to identify and characterise this variant. To address whether this was restricted to this species and to neonatal brain, further western blot analysis was performed and revealed expression of this variant in other species as well as its absence from the adult brain. Using RT-PCR and sequencing methods we identified a previously unreported splice variant of the $GABA_A$ receptor α_3 protein that appears unique to the developing brain.

Poster 42 – Monday 5th December

PHYSICAL EXERCISE IMPROVES AGE-ASSOCIATED COGNITIVE DEFICITS

Dr Daniel Blackmore[1], Mr Richard Wang[1], Dr Frederick Steyn[2], Professor Perry F Bartlett[1]

[1]*Queensland Brain Institute, The University of Queensland,* [2]*School of Biomedical Sciences, The University of Queensland*

Voluntary physical exercise positively effects hippocampal neurogenesis in both young and old animals. In the present study we demonstrate that old (24-month) mice retain a population of neural precursor cells in the dentate gyrus of the hippocampus that can be activated after an optimal period of physical exercise. We also demonstrate that activating these endogenous latent precursor cells correlates to increased neurogenesis, improved cognitive ability and that these changes correlate with an increase in circulating growth hormone (GH).
Young (10-week-old) and old mice were given ad libitum access to a running wheel for increasing periods of time. Young animals demonstrated increased stem cell activation following acute exercise whereas old mice required a prolonged period. The increase in stem cell activation for both ages correlated with an increase in GH. Neurogenesis levels were also significantly increased following an optimised period of exercise. Spatial memory and learning were tested using an active place avoidance (APA) paradigm. Unlike young animals, which were able to learn the location of the shock zone and avoid it, old animals were unable to do so and received significantly more shocks on each day of testing. Following exercise, old mice were found to have improved cognitive function, as indicated by receiving fewer shocks and their performance was comparable to young animals.
Our results demonstrate that exercise can ameliorate cognitive decline in mice of old age. By defining a possible mechanism involved in exercise-mediated cognitive improvements it may be possible to develop effective strategies to combat age-associated cognitive deficits.

Poster 43 – Tuesday 6th December

TDP-43 MEDIATED SYNAPTIC ALTERATIONS IN THE PATHOGENESIS OF TDP-43 PROTEINOPATHIES

Catherine A Blizzard, EE Handley, Dawkins E, Clark R, and Dickson TC

Menzies Institute for Medical Research, University of Tasmania

ALS (Amyotrophic Lateral Sclerosis) and FTD (Frontotemporal Dementia) are the most prevalent TDP-43 proteinopathies, - devastating neurodegenerative disorders pathologically characterised by the presence of large neuronal cytoplasmic aggregates of the RNA binding protein TDP-43. Abnormal localisation and function of TDP-43 is likely to be a critical component of these diseases, as mutation of the *TARDBP* gene (encoding TDP-43), including the A315T mutation, is sufficient to cause familial ALS. The associated neurodegeneration was proposed to result from a toxic gain of TDP-43 function within the cytoplasmic inclusions, or a detrimental loss of normal TDP-43 function from the nucleus. However, resent research indicates that TDP-43 may also play an underappreciated role at the synapse. To probe TDP-43 misprocessing at the synapse we created the TDP-43^{A315T}*: Thy1-YFP* transgenic mouse cross. Our data indicates that there is a significant ($P<0.05$) reduction in total spine density in the motor cortex of *TDP-*43^{A315T}*: Thy1-YFP* transgenic mice relative to *Thy1-YFP* controls that develops between postnatal day 30 and 60. This spine loss significantly precedes excitatory neuronal cell loss, which is only present by day 90. Morphological spine-type analysis revealed that there was a significant impairment in the development of basal mushroom spines in the cortex of *Thy1-YFP::TDP-*43^{A315T} mice compared to *Thy1-YFP* control. This synaptic loss did not alter synaptic protein expression by western blot, but did correspond to a reduction in synaptic transmission as determined by electrophysiology. Our current *in vitro* studies using primary cortical neurons with the *TDP-*43^{A315T} mutation has demonstrated that whilst there was no significant difference in mean dendrite outgrowth or dendrite complexity there was a significant reduction in spine formation. Drawing upon this data we propose that TDP-43 misprocessing may play a pathogenic role in neuronal communication and potentially synaptic plasticity, occurring early in disease progression. Understanding the role that TDP-43 plays in synaptic dysfunction may reveal new therapeutic windows for intervention in TDP-43 proteinopathies.

Poster 44 – Monday 5th December

NEW MOUSE DAP12 MUTANTS FOR THE STUDY OF ALZHEIMER'S DISEASE

Dr Liviu-Gabriel Bodea[1], Prof Harald Neumann[2], Prof Jürgen Götz[1]

[1]*Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), The University of Queensland,* [2]*Institute or Reconstructive Neurobiology, University of Bonn*

Microglia are the resident immune cells of the brain. They respond to changes in the surrounding environment by intracellular pathways involving immunoreceptor tyrosine-dependent activatory motifs (ITAMs) or immunoreceptor tyrosine-dependent inhibitory motifs (ITIMs). Triggering receptor expressed on myeloid cells 2 (TREM2) gene variants were reported as being risk factors for Alzheimer's disease (AD) (Guerreiro et al., 2013; Jonsson et al., 2013). TREM2 relies on the activation of its associated adaptor protein DAP12, an ITAM-bearing molecule. DAP12 was also ranked as a top causal regulator in an AD-specific gain of function network (Zhang et al., 2013).
We first assessed the effect of DAP12 on the uptake of one of the pathological hallmarks of AD, namely the extracellular accumulation of amyloid-β (Aβ). For this, we have generated mouse embryonic stem cell derived microglia lines overexpressing full length or truncated DAP12 (missing the ITAM motif), and showed that the internalisation of Aβ is dependent on the presence on a functional ITAM signalling.
Next, we generated a new mouse model in which the functional tyrosine residues in the ITAM motif of DAP12 were fully or partially mutated into non-phosphorylable phenylalanine residues. For this, we used a CRISPR/Cas9 gene-editing strategy and the resulting offsprings were validated by PCR and Sanger sequencing.
These mice are currently being crossed with fully characterised AD mouse models to understand the role of DAP12 in AD pathology.

Poster 45 – Tuesday 6th December

MYELIN-ASSOCIATED INHIBITOR EXPRESSION FOLLOWING INJURY TO THE PRIMATE NEOCORTEX

Mr Anthony Boghdadi[1], Dr Leon Teo[1], A/Prof James Bourne[1]

[1]*Australian Regenerative Medicine Institute*

Ischemic stroke affecting the primary visual cortex (V1) results in permanent visual impairments. Myelin-associated inhibitors (MAIs): neurite outgrowth inhibitor-A (NogoA), myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), and MAI receptors, Nogo Receptor-1 (NgR1) and paired immunoglobulin-like receptor (PirB), are major inhibitors of regeneration. This study investigates the expression profile of MAIs following focal stroke in 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=16) were used to induce ischemia. Control, 1-day post injury (DPI), 7DPI and 21DPI brains were snap-frozen (n=7) or fixed (n=9) for downstream analysis. Primary areas from control (n=2) and post-stroke human brains (n=2) were used to complement MAI-expression data in marmoset.

Elevated MAI-expression was observed in marmoset V1 (peri-infarct) and adjacent (extrastriate) areas, predominantly at 7DPI, with ligand elevation persisting up to 21DPI. NgR1 was elevated at all time points in extrastriate tissue. Previously unreported, MAG was detected on the majority of neurones (NeuN) in marmoset and human neocortex. Increased NogoA expression was identified on neurones adjacent to lesion core in marmoset V1. MAIs colocalised with GFAP+ marmoset-derived reactive astrocytes *in vitro* and *ex vivo.*

Persistent elevation of NgR1 beyond ischemic core and penumbra, coupled with increased expression of MAIs in the peri-infarct area, suggests a prolonged MAI-receptor interaction post-stroke that is likely detrimental to regeneration. Detection of MAIs on large neuronal populations and reactive astrocytes demonstrates these cell types may play key roles in MAI cellular responses in primates.

Poster 46 – Monday 5th December

DOES PROPRIOCEPTIVE INPUT FROM THE NECK CONTRIBUTE TO THE MODULATION OF SKIN SYMPATHETIC NERVE ACTIVITY SUPPLYING THE LOWER LIMBS OF HUMANS?

Bolton PS[1], Hammam E[2], Macefield VG[2,3]

[1]School of Biomedical Sciences & Pharmacy, University of Newcastle; [2]School of Medicine, Western Sydney University; [3]Neuroscience Research Australia

Purpose: We previously showed dynamic neck displacement is necessary for neck proprioceptor modulation of muscle sympathetic nerve activity to lower limbs of humans (1). In this study we tested whether neck displacements also modulate skin symapthetic nerve activity (SSNA) to the lower limbs.
Methods: SSNA was recorded with tungsten microelectrodes inserted into the common peroneal nerve of 9 subjects lying supine on a special table that fixed their head in space but allowed trapezoidal ramp (8.1 ± 1.2^0/s) and hold (17.5^0 for 53s) or sinusoidal (35^0 peak-to-peak at 0.33-0.46 Hz) horizontal displacement of the body about the head. We simultaneously recorded ECG with surface electrodes and respiration using a strain-gauge transducer around the chest. SSNA was recorded before, during and after trapezoidal and sinusoidal displacements of the body.
Results: Cross-correlation analysis revealed SSNA was not changed by trapezoid body-only displacement to the left or right but was cyclically modulated during sinusoidal angular displacements (median 95% CI; 37.2 ⍰ 8.0%). This was not statistically ($p > 0.05$) different to the cardiac and respiratory modulation at rest (47.1, 18.7-56.3%; 48.6, 28.4-59.3%, respectively) or during sinusoidal displacment (10.3, 6.2 – 32.1%; 26.9, 13.6 – 43.3%, respectively). A matched-pairs analysis showed there was no statistically significant difference ($p>0.05$) between the

respiratory frequency and the sinusoidal displacement, suggesting that the cyclical SSNA modulation was associated with respiratory modulation.
Conclusion: This study suggests proprioceptors in the neck do not contribute to the physiological modulation of SSNA.
1) Bolton et al (2014) Exp Br Res 232:2262-2271

Poster 47 – Tuesday 6th December

GLYCOLYSIS IS REDUCED ICTALLY IN THE ACUTE FLUOROTHYL MOUSE SEIZURE MODEL

Karin Borges[1], Tanya McDonald[1]

[1]University of Queensland

It is generally thought that there is increased cerebral blood flow and hypermetabolism during seizures. Here we investigated to which extent glycolysis and the tricarboxylic acid (TCA) cycle are affected. Ten minutes before induction of fluorothyl-induced seizures, we injected 558 mg/kg [U-$^{13}C_6$]-glucose (i.p.). After 5 min generalized seizure activity, mice were sacrificed. In another cohort, mice were injected with [U-$^{13}C_6$]-glucose immediately following five min seizure activity and sacrificed 15 minutes later. The % enrichment of ^{13}C in major metabolites from the hippocampal formation was determined. Activities of glycolytic and TCA cycle enzymes were measured using spectrophotometry. Immediately after 5 min seizure activity, reductions in % enrichment of ^{13}C were only found in lactate, alanine and GABA by 27-33% ($p<0.001$ two-way ANOVAs and post tests) and the activity of phosphofructokinase was lowered by 30% ($p<0.05$). This suggests that glycolysis was reduced. Post-ictally, 44-91% reductions in % enrichment were found in all metabolites, including the TCA cycle metabolites citrate, succinate, fumarate and malate as well as the amino acids aspartate, glutamate and glutamine ($p<0.05$). At this time point the activity of pyruvate dehydrogenase was reduced by 40% ($p<0.05$), with a 1.9-fold increase in the phosphorylation of this enzyme at the S232 site,indicating impaired entry into the TCA cycle
In the acute fluorothyl mouse model glycolysis is reduced during seizures, while the TCA cycle is impaired afterwards. It is likely that the impaired brain metabolism during and after seizures cannot meet the demand for ATP, which would contribute to post-ictal depression in the brain.

Poster 48 – Monday 5th December

MUSCULAR DOMINANCE UNDERLYING PAG TRIGGERED VOCAL MOTOR EXPRESSION

Borloo T*, Lo M*, Silburn PA, Subramanian HH

Queensland Brain Institute, Asia-Pacific Center for Neuromodulation, University of Queensland, St. Lucia, QLD, Australia 4072.

Muscular dominance underlies vocal expression both verbal and non-verbal in mammals. For example, thyroarytenoid muscle function dominates over cricothyroid during low pitch singing. In spasmodic dysphonia, neural dissociation specific to laryngeal, diaphragm and abdominal muscles impact on motor components of speech. In such conditions suppression of one particular muscle tends to limit the overall vocal register. Neurochemical microstimulation of midbrain periaqueductal gray (PAG) in decerebrate cat elicits different types of vocalization, as mew, howl and cry[1]. They are of varied durations and posses distinct motor orchestration of laryngeal, respiratory and abdominal muscles[1]. Using cat muscle EMGs obtained, we intended to find whether specific muscles within the laryngeal and respiratory system play a dominant role in orchestrating specific vocal calls. We used root mean square (RMS), frequency, slope and wavelet analysis of cricothyroid, thyroarytenoid, diaphragm and abdominal muscle EMGs to infer on the dominance of specific muscle during vocalization. We found that external oblique plays a dominant role in the orchestration of a mew, while thyroarytenoid muscle for the cry. RMS curves for different vocalizations show a range of temporal activations of the diaphragm. Power density spectra of the EMGs for the laryngeal and abdominal muscles illustrate differences between mew and cry. We present quantitative modeling of EMG for examining muscle dominance underlying vocal motor control.

* contributed equally to the study
Subramanian HH, Arun M, Silburn PA and Holstege G (2016). Motor organization of positive and negative emotional vocalization in the cat midbrain periaqueductal gray. *J. Comp. Neurol.* 524:1540-57

Poster 49 – Tuesday 6th December

EFFICIENT GLIA-DRIVEN NEUROREGENERATION FOLLOWING EXTRACELLULAR ATP INJURY IN ZEBRAFISH RETINA

Dr Alice Brandli[1], Ms Steffi Dudczig[2], Prof Peter Currie[3], Dr. Patricia Jusuf[1]

[1]Department of Anatomy and Neuroscience, University of Melbourne, [2]School of Bioscience, University Of Melbourne, [3]Australian Regenerative Medicine Institute, Monash University

Loss of light detecting photoreceptor retinal neurons leads to blindness. A novel approach to vision restoration is the stimulation of endogenous retinal glial cells to produce photoreceptors, which has the potential to reverse vision loss. We used the highly regenerative zebrafish model to characterise the regenerative events following extracellular ATP (a potent activator of glia and photoreceptor toxin) in the retina.

ATP or PBS control was injected into the vitreous of adult Tg(*GFAP:GFP*) zebrafish (mature glia marker) or Tg(*PCNA:GFP*) zebrafish (proliferating marker) zebrafish. Animals were culled between 1-7 days post-injection (dpi). The time course of cell death (TUNEL staining), proliferation (PCNA) and the cellular source of proliferation (GFAP) was quantified.
ATP injections (20 mM) caused an increase in cell death (TUNEL+) in the outer retina and disruption of photoreceptor layers (formation of rosettes) from 1 dpi (t-test; $p < 0.001$ PBS injected vs. 20 mM ATP injections). Average proliferative PCNA:GFP+ cell counts increased between 2-5 dpi (range: 4-10 cells/section), peaking at 4 dpi (10 cells/section). At 3 dpi, proliferative PCNA:GFP+ cells were observed adjacent or within rosettes in all retinal layers with the vast majority (74%) found in the inner nuclear layer (INL). All of these INL proliferative cells co-labelled with GFAP:GFP suggesting that regenerating cells are glia-derived.
Hence, ATP causes selective photoreceptor loss and glia differentiation in zebrafish. Further studies using this model will enable comparative cross-species studies (lower vertebrate vs. higher vertebrate) to identify reprogramming genes essential for production of glial-derived photoreceptors, which can be reactivated in mammals.

Poster 50 – Monday 5th December

MECHANICAL PERCEPTION IMPROVED AND NEURAL JITTER REDUCED VIA PROXIMALLY APPLIED SUBSENSORY ELECTRICAL NOISE STIMULATION

Dr Paul P Breen[1], Dr Yossi Buskila[1], Prof Vaughan G Macefield[1,2]
[1]The MARCS Institute, Western Sydney University, [2]School of Medicine, Western Sydney University,

Loss of somatosensory function is detrimental to quality of life and is related to the loss of functional ability. Previous work has demonstrated that the proximal application of subsensory electrical noise stimulation (SENS) can enhance sensitivity to a distal mechanical stimulus. This paper presents results from a series of experiments undertaken in an attempt to understand the underlying mechanism generating the observed improvements in vibrotactile perception.
The first experiment assessed vibration perception thresholds at the fingertip with and without the proximal application of SENS to establish if similar enhancements of sensory perception can be observed in this paradigm. A second experiment used microneurography to record neural traffic in individual neurons and determine the effect of proximal SENS. Finally; a computer model of the experimental axon was created to delve further into the findings of the previous experiments.
Vibration perception was increased with all levels of SENS, significantly so at 40 and 60% of the SENS perceptible level ($p<0.05$). Microneurography demonstrates that ISI jitter is significantly reduced in line with increases in the SENS amplitude, but hits a limit where presumably SENS generates incoherent action potentials. Together the vibration perception and microneurography results suggest that a particular level of SENS will produce an optimal reduction in ISI jitter that is experienced as improved sensory perception. Above this level ISI jitter is increased and perception is reduced. Modelling results suggest that this may be explained by the unequal impact of subthreshold membrane hyperpolarization (beneficial) and depolarization (not beneficial).

Poster 51 – Tuesday 6th December

NOVEL MOUSE MODEL OF AN ALS-ASSOCIATED PFN1 MUTATION

Miss Merryn Brettle[1], Miss Holly Stefen[2], Miss Josephine Chan[2], Miss Aleksandra Djordjevic[2], Dr Fabien Delerue[1], Dr Yazi Ke[4], Dr Thomas Fath[2], Prof Lars Ittner[1]

[1]Dementia Research Unit UNSW, [2]Neurodegeneration and Repair Unit UNSW, [3]Trangenic Animal Unit UNSW, [4]Motor Neuron Disease Unit, [5]Neuroscience Research Australia

Amyotrophic lateral sclerosis 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 profilin 1 have been identified as a rare cause of familial ALS, but how these mutations cause ALS is unknown. We have developed a novel mouse model to elucidate the role that $PFN1^{C71G}$ plays in ALS. Expression of V5-tagged $PFN1^{C71G}$ was targeted to α-motor neurons in the spinal cord. Initial data shows V5- $PFN1^{C71G}$ expression in the anterior horn of the neural tube starting from embryonic stages in transgenic mice. Motor testing shows that transgenic mice have progressive motor deficits on RotaRod commencing at 2 months of age. This novel mouse model of $PFN1^{C71G}$ 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.

Poster 52 – Monday 5th December

STRESS-INDUCED PROLACTIN SIGNALLING IN THE MALE MOUSE.

Dr Stephen Bunn[1], Ms Siobhan Kirk[1], Prof Dave Grattan[1]

[1]Centre for Neuroendocrinology, University Of Otago

Prolactin release from the anterior pituitary is regulated by a negative feedback loop, in which it activates the tuberoinfundibular dopaminergic (TIDA) neurons in the arcuate nucleus, thus releasing dopamine into the median eminence to suppress further prolactin secretion. By measuring the phosphorylation of Signal Transducer and Activator of Transcription-5 (STAT5) we have shown that exogenous prolactin rapidly signals to the arcuate nucleus and median eminence. In this study we have investigated if stress-induced prolactin

secretion results in a similar response. Male mice (n=15) were treated with either bromocriptine (10µg) or saline for 24h and then exposed either to restraint stress or home cage environment for 15 minutes prior to euthanasia using pentobarbitone and transcardiac perfusion (4% paraformaldehyde). Hypothalamic sections were then processed for immunohistochemistry. The arcuate nucleus showed a significant increase in phospho-STAT5 in its ventral regions when exposed to restraint stress ($p<0.05$). This response was abolished by bromocriptine, indicating that it was prolactin dependent ($p<0.05$). Restraint stress also resulted in a prolactin-dependent increase in phospho-STAT5 staining in the median eminence. As observed in response to exogenous prolactin administration this latter staining was non-nuclear with a distinctive granular distribution. Dual-immunolabelling with either vimentin or β-III tubulin (markers for tanycytes or neurons) indicated that the phospho-STAT5 was located within neuronal processes. Although the specific neuronal type involved has yet to be identified these findings indicate that neuronal elements within the median eminence respond to stress-induced elevation in prolactin, suggesting that this event may be physiologically meaningful.

Poster 53 – Tuesday 6th December

ALTERED ATTENTION IN THE NEUROLIGIN-3 GENETIC MOUSE MODEL OF AUTISM SPECTRUM DISORDER

Dr Emma Burrows[1], Mr Carlos May[1], Mr Thomas Hill[1], Professor Anthony Hannan[1]

[1]*The Florey Institute Of Neuroscience And Mental Health*

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by social communication impairments and repetitive and restrictive behaviour. Mice containing the ASD-associated R451C mutation in the gene coding for the synaptic adhesion protein Neuroligin-3 (NL3) exhibit impaired reciprocal social interactions as well as repetitive and restrictive behaviours. Individuals with ASD also exhibit a variety of comorbid traits such as attentional impairments, which adversely impact on their quality of life. Investigations into these secondary traits in genetic animal models may provide valuable information pertinent to root biological underpinnings of ASD. We aimed to expand the behavioural characterization of $NL3^{R451C}$ mice, assessing behavioural flexibility and attention using automated touchscreens. $NL3^{R451C}$ mice were assessed for impairments in 2-choice visual discrimination and reversal learning. $NL3^{R451C}$ mice were slower to learn a new reward-stimuli association in the reversal learning paradigm, indicating impaired behavioural flexibility. Mice were assessed for attentional phenotypes in the 5-choice serial reaction task (5CSRT) and continuous-performance test (CPT). NL3 mice were found to be less likely to spot stimuli, more accurate when selecting stimuli, slower at making responses and also showed an increased preference for reward. Although attentional deficits are frequently reported in clinical autism, this is the first time an ASD-associated gene mutation has been demonstrated to cause an attentional phenotype. The verification of these specific behavioural differences relevant to the symptoms of ASD further advances the translational validity of the $NL3^{R451C}$ mouse and provides a model for future study into the causes and treatment of ASD and its co-morbid complications.

Poster 54 – Monday 5th December

CONSISTENCY IN THE EVOLUTION OF CHOICE BEHAVIOUR ACROSS EPOCHS OF LEARNING AND ADAPTATION TO CHANGE

Mr Thomas Burton[1], Dr Atomu Sawatari[1]

[1]*The University Of Sydney*

Goal-directed learning, informed decision making and behavioural flexibility are critical for survival and prosperity and must maintain robust functionality in dynamic and uncertain situations. Though traditional approaches have given much insight into understanding the neural mechanisms underlying learning and flexible decision making, they do not necessarily consider the complexity in which naturalistic behaviour is generated and experienced.
Using a more complex and naturalistic behavioural testing setting, we have developed a novel approach for investigating a range of cognitive processes in mice. Adult male C57BL/6 mice were housed in groups of 4 in an IntelliCage and were required to learn one of either two instrumental tasks to gain access to water in this home cage setting: a Visual cue Discrimination [VD] task or a Response Discrimination [RD] task. Once an animal reached acquisition criterion the task contingencies were changed in one of either two ways: an Extradimensional Shift [EDS; between-modality] or a Rule Reversal [RR; within-modality].
Change point analysis executed on individual cumulative records of performance and behaviour revealed that mice consistently exhibit distinct phases of learning and adaptation to changes in task contingencies. For example, 3 robust phases emerge in sequence after VD-RR which we term *Perseverance*, *Presolution* and *Solution*. Furthermore, transitions between these phases appear abrupt and even step-like in nature. Our more complex and naturalistic approach allows for a novel and highly detailed examination of the evolution of choice behaviour across entire epochs of instrumental learning, uncertainty and adaptation to change.

Poster 55 – Tuesday 6th December

ESTABLISHING A NOVEL MODEL TO ASSESS NEUROBIOLOGICAL AND FUNCTIONAL CONSEQUENCES OF TRAUMATIC BRAIN INJURY IN INFANTS

Dr Nicole Bye[1], Ms Alisa Turbic[2], A/Prof Ann Turnley[2]

[1]*Division of Pharmacy, Utas,* [2]*Department of Anatomy and Neuroscience, University of Melbourne*

Brain development continues until late adolescence, meaning that traumatic brain injury (TBI) perturbs different developmental milestones and cellular processes depending on age at injury. Therefore, it was our objective to develop a novel mouse model of TBI, performed at postnatal day (P) 7 (~equivalent to human infant 0-6 months) when the forebrain is beginning to myelinate, to allow us to define functional and histopathological consequences of infant TBI. A controlled cortical impact device was used to produce a regulated mechanical deformation to the intact skull over the left motor cortex of P7 mice. Mice were killed at 1,3 or 7d post-TBI and immunohistochemical analyses were performed to assess multiple pathologies/regenerative processes. Social behaviours were assessed for up to 4 months post-TBI. In the injured cortex, we detected FluoroJade+ degenerating neurons at 1d post-TBI, BBB deficiencies to 3d, and an accumulation of GFAP+ astrocytes at 7d ($P<0.05$). Oligodendrocyte development was also perturbed, with increased proliferation of PDGFRα+ progenitors ($p<0.05$) but a decrease in mature CC1+ oligodendrocytes ($p<0.01$) within 1w of injury. At 3-4 months, mice that received TBI as pups developed delayed behavioural deficits in Exploration ($p<0.05$) and Social Following ($p<0.01$), but not in other behaviours such as Social Sniff. These data are among the first to identify neurobiological consequences of infant TBI. Our continuing studies in this area will provide essential knowledge of mechanisms that lead to ongoing deficit following infant brain injury and provide a valuable resource for translational research, uncovering new targets for design of potential new therapies.

Poster 56 – Monday 5th December

BLOOD-BRAIN BARRIER GENE EXPRESSIONS CHANGED IN SCHIZOPHRENIA AND BIPOLAR DISORDER ASSOCIATED WITH IMMUNE AND STRESS PATHWAYS

Miss Helen Cai[1,2,3], Dr Vibeke Catts[1,2,3], Dr Maree Webster[4], Professor Cynthia Shannon Weickert[1,2,3]

[1]Schizophrenia Research Institute, [2]Schizophrenia Research Laboratory, Neuroscience Research Australia, [3]School of Psychiatry, University of New South Wales, [4]Stanley Medical Research Institute

Abnormal regulation of immune and stress pathways are apparent in schizophrenia and bipolar disorder. We investigated the mRNA expression of genes important for blood-brain barrier (BBB) integrity, which may be altered in these individuals especially if in a high inflammation/stress state. We measured the expression of breast cancer resistance protein (ABCG2), interferon-induced transmembrane protein (IFITM), intercellular adhesion molecule (ICAM1), VE-cadherin (CDH5) and occludin (OCLN) with RT-qPCR in the Stanley Array Cohort (AC) of 35 schizophrenia/35 bipolar disorder (BPD)/35 controls. We found ABCG2 mRNA downregulated ($F(2,90)=4.740$, $p=0.011$) and IFITM mRNA upregulated ($F(2,89)=5.703$, $p=0.005$) in BPD and schizophrenia compared to controls. There were no diagnostic differences in ICAM1, OCLN or CDH5 mRNA levels. We previously clustered the AC into high and low inflammation/stress biotypes based on pro-inflammatory and glucocorticoid receptor mRNA expression in the dorsolateral prefrontal cortex. The high inflammation/stress BPD cases had elevated CDH5 ($F(4,82)=3.245$, $p=0.016$) and ICAM1 ($F(4,75)=3.690$, $p=0.009$) relative to low inflammation/stress BPD cases and controls and were also increased in high inflammation/stress schizophrenia cases compared to controls ($p<0.05$). ICAM mRNA was elevated in high inflammation/stress schizophrenia cases relative to low inflammation/stress schizophrenia cases ($p=0.05$). High inflammation/stress schizophrenia cases had increased IFITM ($F(4,83)=9.263$, $p<0.001$) and decreased ABCG2 ($F(4,85)=8.226$, $p<0.001$) compared to low inflammation/stress schizophrenia cases and controls. These results implicate the BBB in schizophrenia and BPD and may be exacerbated in individuals with an elevated inflammation/stress state. Subset specific differences within a diagnostic category and also across diagnoses may contribute to heterogeneity found within and across psychiatric illnesses.

Poster 57 – Tuesday 6th December

COLONIC NOCICEPTIVE PATHWAYS ARE POTENTLY ACTIVATED BY CIGUATOXIN WITH GREATEST EFFICACY IN A MODEL OF CHRONIC VISCERAL HYPERSENSITIVITY

Miss Ashlee Caldwell[1], Dr Joel Castro[1], Dr Luke Grundy[1], Dr Andrea M Harrington[1], Dr Sonia Garcia-Caraballo[1], Mrs Jessica Maddern[1], A/Prof Grigori Y Rychkov[1], Professor Richard J Lewis[2], Dr Irina Vetter[2], A/Prof Stuart M Brierley[1,3]

[1]Visceral Pain Group, Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, The University of Adelaide, SAHMRI, [2]Institute for Molecular Bioscience, The University of Queensland, [3]Visceral Pain Group, Flinders University

Pain is detected by primary sensory afferents projecting from peripheral tissues to the dorsal horn of the spinal cord, via activation of ion channels, including voltage gated sodium (Nav) channels. Ciguatoxin (P-CTX-1) is a potent Nav channel activator, which upon ingestion of contaminated fish causes intense abdominal pain in humans.
Utilising an *ex-vivo* colonic afferent preparation and calcium imaging of isolated colonic DRG neurons from both healthy mice and mice with chronic visceral hypersensitivity (CVH), we used P-CTX-1 to investigate the neural mechanisms and the NaV channels underlying visceral pain.
In *ex-vivo* colonic afferent studies, P-CTX-1 potently activated 74% of colonic nociceptors as well as activating a population of 'silent nociceptors'. Correspondingly, *in vivo* intra colonic administration of P-CTX-1 caused significant activation of nociceptive signalling pathways within the dorsal horn of the thoracolumbar spinal cord, as well as altering spontaneous behavioural responses ($p\leq0.01$). Calcium imaging studies showed 83% of healthy colonic DRG neurons were activated by P-CTX-1, while 100% of CVH colonic DRG neurons responded to the toxin ($p\leq0.05$). A larger proportion of the P-CTX-1 response was sensitive to tetrodotoxin (TTX) in CVH colonic DRG neurons compared to healthy (37.2 Vs 14.9% $p\leq0.01$).
Overall, these results suggest P-CTX-1 is a novel tool for the investigation of the mechanisms underlying visceral pain and indicates a significant role for TTX sensitive Nav channels in neuronal excitability. These results also suggest that NaV channel are an essential component of the hyperalgesia and allodynia associated with CVH.

Poster 58 – Monday 5th December

INNERVATION AND CHEMICAL TAXONOMY OF GASTRIC ENTEROENDOCRINE CELLS

Dr Brid Callaghan[1], Ms Billie Hunne[1], Ms Linda Fothergill[1], Mr Mitchell Ringuet[1,2], Ms Josiane Fakhry[1], Prof John Furness[1,2]

[1]Department of Anatomy and Neuroscience, [2] Florey Institute of Neuroscience and Mental Health

The stomach is a major center for the integration of responses to a meal. It detects volume, nutrient content and luminal chemistry. Luminal receptors are expressed by gastric enteroendocrine cells (EEC) that are in contact with the gastric content and which release hormones that signal to vagal afferent neurons. In turn, efferent nerves control secretion from at least some EEC. Despite their obvious importance, there is no inventory of gastric EEC or the afferent and efferent neurons that connect with them. To address this, we are characterising the EEC taxonomy of the rat stomach and measuring the proximity of populations of nerve fibers. Specifically we have utilised triple label immunohistochemistry of cryosections of antral and oxyntic mucosa with antibodies to ghrelin and gastrin (for EEC) and VIP, CGRP or VAChT, for axons. Gastrin cells were observed in the base of the antral mucosa with an open type morphology, ghrelin cells were distributed throughout the length of the antral and oxyntic mucosa with a closed morphology. We observed co-storage of ghrelin and gastrin in EEC in only 0.02% of 300 EEC counted. Nerve fiber proximity to each of the EEC populations was measured in confocal images using ImageJ. The % of gastrin cells within 5µM of the fibres was 50% for VIP, 40% for VAChT and 35% for CGRP, for ghrelin 30% were within 5µM of VIP, 24% of VAChT and 22% of CGRP axons. Thus both of these EEC cell types is innervated, but with different patterns of nerve supply.

Poster 59 – Tuesday 6th December

EVIDENCE THAT A NOVEL AGONIST OF GHRELIN RECEPTORS PARTICIPATES IN NEURAL CONTROL OF THE COLORECTUM

Dr Brid P Callaghan[1], Dr Ruslan V Pustovit[1], Ms Billie Hunne[1], Ms Nicole F Kerr[1], Mr Mitchell Ringuet[1,2], Ms Josiane Fakhry[1], Prof John B Furness[1,2]

[1]Department of Anatomy and Neuroscience, University Of Melbourne, [2]Florey Institute of Neuroscience and Mental Health

Agonists of the ghrelin receptor, GHSR1a, cause colorectal propulsion and defecation in mice, rats, dogs and humans, through actions on nerve cells expressing GHRSR1a within the lumbosacral defecation centres. However, the only known natural agonist of GHRSR1a, ghrelin, appears to be absent from the CNS. Here we describe experiments to test whether the pharmacological observations are indicative of a physiological role of ghrelin in the control of defecation. Activation of defecation pathways by water avoidance stress (WAS) in conscious rats caused defecation that was antagonized by the GHSR1a antagonist, YIL781. In rats in which the spinal cord was severed 5 weeks before WAS, the numbers of pellets expelled in the first 15 min of WAS was reduced from 9 ± 0.5 (sham, n=6) to 3.8 ± 0.2 (SCI, n=6). Conversely, the effectiveness of the GHSR1a agonist, HM01, to cause defecation was doubled in SCI compared to sham animals. Three different antibodies to ghrelin, and an antibody to des-acyl ghrelin, were effective in revealing ghrelin in gastric endocrine cells, but even in concentrations 10 times those effective in the stomach, they failed to reveal ghrelin in nerve terminals in the spinal cord. The effectiveness of GHSR1a agonists in causing defecation, supersensitivity to agonists after SCI (suggesting denervation sensitivity), and the reduction of defecation in response to a behavioural stimulus by a GHSR1a antagonist, all suggest a natural ligand of GHSR1a is involved. However, ghrelin cannot be detected in the lumbosacral defecation centre. The identity of the natural ligand remains mysterious.

Poster 60 – Monday 5th December

CALCIUM-IMAGING TECHNIQUES IN THE MOUSE RETINA

Dr Morven Cameron[1], Professor John Morley[1], Dr Yossi Buskila[1]

[1]Western Sydney University

Calcium-imaging is a sensitive method for monitoring calcium dynamics during neuronal activity and is one of the most commonly used techniques in neuroscience today. However, the technique has never been commonly used in the retina due to the exquisite sensitivity of this tissue to visible light, and the difficulty loading retinal neurons with calcium dyes. With optical breakthroughs such as two-photon microscopy, and the development of new techniques that allow ubiquitous staining of retinal neurons, calcium-imaging in the retina has expanded considerably. However, the prolonged dissection and loading time of retinal tissue curtails experimental time, as tissue is typically maintained for only 6–8 hours after extraction. Using a recently introduced chamber, the Braincubator™, we tested calcium dye responses following long incubation periods after cell loading in wholemount retinae. We compare two loading techniques: 1). bolus injection of AM dyes beneath the inner limiting membrane (INL), 2). AM loading following digestion of the INL with the enzyme papain. We show that calcium dyes remain within cells and are fully functional >24 hours after loading. Moreover, the calcium dynamics recorded >24 hrs were not statistically different to those recorded in fresh tissue incubated for <4 hrs. These results indicate that long exposure of calcium AM dyes to the intracellular cytoplasm did not alter the intracellular calcium concentration, the functional range of the dye, or viability of the neurons. These methods will extend experimental time for those using acute neuronal tissue, and reduce the number of animals required to complete experimental goals.

Poster 61 – Tuesday 6th December

ROTENONE MIMICS THE IMPACT OF THE K369I TAU MUTATION IN A MODEL OF NEURODEGENERATIVE DISEASE

Mr Max Mo[1], Miss Miranda Mathews[2], Miss Victoria Tung[2], Dr. Daniel Johnstone[1], Dr Aaron Camp[2]

[1]*Department of Physiology, University Of Sydney,* [2]*Discipline of Biomedical Science, University Of Sydney*

Objective: Many neurodegenerative tauopathies including Progressive Supranuclear Palsy (PSP), lack validated animal models. To characterize the suitability of two previously used models of neurodegenerative disease for the study of PSP, 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 entire 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.

Poster 62 – Monday 5th December

50B11 CELLS AS A PERIPHERAL SENSORY NOCICEPTIVE NEURON MODEL FOR NEUROTROPHIN SIGNALLING

Canlas JJ [1], Martin A[2], Keating D[2], Haberberger RV[1], Matusica D[1]

[1]Pain and Pulmonary Neurobiology, Anatomy and Histology, Centre for Neuroscience, Flinders University, Adelaide, SA, Australia.
[2]Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, SA, Australia; South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia.

Stimulation and sensitization of nociceptive neurons with cell bodies in dorsal root ganglia (DRG) play a critical role in the development of chronic and neuropathic pain.
Investigations of DRG neurons are limited by the absence of a suitable cellular high-throughput model capable of representing individual cell types found in DRG. The aim of this study was to establish whether an immortalized rat embryonic DRG cell (50B11), can be selectively differentiated into NGF-dependent peptidergic and GDNF-dependent non-peptidergic nociceptors. We used q-RT-PCR, Western blotting, In-Cell Western blots, immunohistochemistry and calcium efflux flow cytometry to determine the nociceptive characteristics of differentiated 50B11 cells via determination of markers at transcriptional (TRPV1, P2X3 and TrkA, n = 5) and translational levels (TRPV1, CGRP, P2X3, TrkA, p75NTR, and GFRα, n = 5; immunohistochemistry, CGRP, SP, P2X3, and IB4, n = 5). Functional characteristics were investigated as response to ATP and capsaicin in 50B11 cells differentiated with forskolin in combination with NGF or GDNF. Our results show that NGF and GDNF treatment of 50B11 cells differentially regulate the cells to develop into a peptidergic (TRPV1 expressing, capsaicin-responsive) or non-peptidergic (P2X3 expressing, ATP-responsive) subtype with morphological features of peripheral sensory neurons. Our data indicate that the 50B11 cells – depending on culture conditions – have the capacity to differentiate into both peptidergic and non-peptidergic nociceptive neurons. This suggests that the cell line is a valuable tool for the study of nociceptive signaling events modeling peripheral nociceptors.

Poster 63 – Tuesday 6th December

KNOCKDOWN OF MEDIAL HYPOTHALAMIC OREXIN ATTENUATES HYPERTENSION IN ADULT SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

Mr Bruno W Dampney[1], **Dr Pascal Carrive**[1]
[1]*Unsw Australia*

The neuropeptide orexin activates the sympathetic system and contributes to the regulation of blood pressure as part of its role in the control of arousal during wakefulness and motivated behaviour. Recent work suggests that an upregulated orexinergic system in the medial hypothalamus contributes to the hypertensive phenotype of the spontaneously hypertensive rat (SHR). Consistent with this, cytotoxic lesions of the medial hypothalamus reduce baseline blood pressure in the SHR. AIM: To knockdown the expression of orexin in the medial hypothalamus to determine how important orexin from the medial hypothalamus is in the hypertensive phenotype of the SHR. METHODS: SHR (10-14 weeks old, n=15) and Wistar Kyoto rats (WKY, n=15) were implanted with telemetric probes and bilaterally injected in the medial hypothalamus with an adeno-associated virus (AAV) expressing an shRNA against orexin (Orx). Mean blood pressure (MAP) was recorded day and night weekly for 1 month. RESULTS: A significant interaction between strain and treatment (p = 0.041) was found for baseline MAP recorded in the morning period (7am-1pm, the period when rats are most at rest) when comparing before and 1 month after injection. The drop in the SHR was 10 mmHg and significant (144vs134 mmHg, p<0.0001). In WKY rats the drop was 4 mmHg and non significant (97vs93 mmHg). Immunostaining for orexin and NeuN confirmed a reduction in medial hypothalamus orexin expression without sign of neuronal

damage. CONCLUSION: The upregulation of orexin in the medial hypothalamus contributes to elevated baseline blood pressure in the SHR during the rest period

Poster 64 – Monday 5th December

SYNAPTIC CHANGES INDUCED BY MELANOCORTIN SIGNALING: ROLE IN MEMORY, NOCICEPTION AND MOOD DISORDERS.

Vanni Caruso[1], Malin C Lagerström, Pawel K. Olszewski, Robert Fredriksson, Helgi B. Schiöth

[1]University of Tasmania, [2]University of Uppsala, [3]University of Uppsala, [4]University of Waikato

The melanocortin system has a well-established role in the regulation of energy homeostasis, but there is growing evidence of its involvement in memory, nociception, mood disorders and addiction. We focus on the role of melanocortin 4 receptor and provide an integrative view of the molecular mechanisms leading to melanocortin-induced changes in synaptic plasticity within these diverse physiological systems. We also highlight the importance of melanocortin peptides and receptors in chronic pain syndromes, memory impairments, depression and drug abuse, and the possibility of targeting them for therapeutic purposes.

Poster 65 – Tuesday 6th December

A NOVEL, QUANTITATIVE ASSESSMENT TOOL TO MEASURE FUNCTIONAL CHANGE IN NEUROPSYCHIATRIC DISEASES

Ms Verity Chadwick[1], Dr Amit Lampit[1], A/Prof Michael Valenzuela[1]

[1]University Of Sydney

Neuropsychiatric diseases are frequently diagnosed in late stages when neurological damage is already widespread, because many diseases lack quantitative assessment measures of functional change. This presents a significant problem when assessing the effectiveness of interventions, as the current gold standard is self-report questionnaires that are prone to reporter bias and insensitive to subtle change. Animal models in behavioural neuroscience measure and compare functioning through automated analysis of video footage, but due to privacy concerns this has not yet been carried out in human populations. Our group is conducting a novel exploratory study assessing function in populations with prodromal dementia using modified Kinect cameras to analyse participant's spatial patterns. The sensors reduce image data in one fifteenth of a second to X,Y coordinates for movement to be tracked against the house's floor plan over a period of seven days. Machine-learning algorithms can pick up characteristic behaviours of the disease, such as pacing, disrupted circadian rhythm, and decreased time spent grooming or cleaning, and compare current behaviour to participant's past 'healthy' behaviour, where no cognitive symptoms of dementia were evident. This study is the first to measure real-life behaviour and has the potential to be applied to a number of neuropsychiatric disorders as an empirical measure of functional decline or improvement.

Poster 66 – Monday 5th December

THE EFFECT OF TPM3.1 OVEREXPRESSION ON SYNAPTIC FUNCTION

Ms Chanchanok Chaichim[1], Dr Thomas Fath[2], Dr John Power

[1]Translational Neuroscience Facility, School of Medical Sciences, UNSW Australia, [2]Neurodegeneration and Repair Unit, School of Medical Sciences, UNSW Australia

Alzheimer's disease (AD) is a disorder characterised by increased accumulation of amyloid β (Aβ) in the brain. Previous studies suggest that soluble Aβ oligomers activate a signalling pathway that leads to activation of cofilin, depolymerizing actin, thus causing collapse of dendritic spines and decline in synaptic function. The actin-binding protein tropomyosin Tpm3.1 is known to prevent cofilin binding to actin. We had previously found increased expression of Tpm3.1 at synapses in the brains of AD model mice, potentially to compensate for synaptic impairment caused by Aβ. Here we recorded hippocampal field excitatory postsynaptic potentials (fEPSPs) in brain slices from transgenic mice expressing human Tpm3.1 and littermate controls to examine whether Tpm3.1 overexpression alters excitatory synaptic function or long-term synaptic plasticity. Horizontal brain slices (400 µm) were prepared from 42-56 d transgenic and control mice according to standard procedures approved by the UNSW Animal Care and Ethics Committee. fEPSPs were recorded via a wire electrode positioned in CA1 stratum radiatum and evoked by Shaffer collateral stimulation. There were no apparent differences in either the stimulus-response function or the paired pulse response suggesting that overexpression of Tpm3.1 had little or no effect on basal synaptic function. The fEPSP enhancement evoked by high frequency stimulation (100 Hz 1s) was greater ($p = 0.04$; RM-ANOVA) in Tpm3.1 transgenic mice ($n = 16$) than controls ($n = 15$). The facilitating effect of Tpm3.1 overexpression on long-term synaptic plasticity provides support for Tpm3.1 as potential target in the treatment of AD

Poster 67 – Tuesday 6th December

TOWARDS A CELLULAR-REOLUTION CONNECTOME OF THE PRIMATE BRAIN: SHARING DATA ON CORTICOCORTICAL CONNECTIVITY THROUGH AN OPEN ACCESS WEB PLATFORM

Mr Jonathan Chan[1], Dr Piotr Majka[1,2,3], Prof Partha Mitra[2,4], Dr Daniel Ferrante[4], Mr Shi Bai[1], Prof Marcello Rosa[1,2]

[1]Monash University, [2]Australian Research Council, Center of Excellence for Integrative Brain Function, [3]Necki Inst. of Exptl. Biol., [4]Cold Spring Harbor Lab

The marmoset (*Callithrix jacchus*) is an emerging animal model for large-scale attempts to understand primate brain connectivity. Here we describe an open access, web-based platform for sharing data from the neuroanatomical experiments on this species. The initial release (marmoset.braincircuits.org) focuses on corticocortical connectivity and includes 37 retrograde tracer injections in 18 animals.

The site gives access to images of Nissl-stained sections with overlays showing the location of injection sites and labelled neurons, providing a co-registered atlas for every animal. Our approach is to store a single multi-resolution data stream, which is unpacked in real time at the desired resolution, using a customized Java-based Djatoka image server (https://sourceforge.net/projects/djatoka/). Our web-based viewer allows the user zooming functionality while freely overlaying multiple layers of information, without the installation of software.

Identification of likely areas is based on the registration of a subject's data to the marmoset brain template generated using (Paxinos et al., 2012; The Marmoset Brain in Stereotaxic Coordinates; ftp://ftp.space.intersect.org.au/neural/). Comparison between the results of the automated and human-based processing reveals that the centre of the injection sites can be reconstructed, on average, to within 0.6mm, (range 0-1.4mm). By laying the foundation of an open access resource, we intend to enable comparison and visualization of large data sets, allowing in turn integration and analysis of results from many cases. The applicability to archival materials may reduce the number of additional experiments required to produce the first detailed cortical connectome of a primate brain.

Poster 68 – Monday 5th December

MATERNAL SMOKING IN MICE ALTERS MARKERS OF MITOPHAGY IN OFFSPRING FOLLOWING HYPOXIC-ISCHEMIC BRAIN INJURY

Mr Yik Lung Chan[1], Dr Sonia Saad[2], Dr Nicole Jones[3], Dr Hui Chen[1]

[1]School of Life Sciences, Faculty of Science, University of Technology Sydney, [2]Renal Medicine, Kolling Institute of Medical Research, Royal North Shore Hospital, [3]Department of Pharmacology, School of Medical Sciences, University of New South Wales

We have previously shown that maternal smoking during pregnancy can change mitochondrial functional markers in the offspring brain in adulthood. Maternal smoking is also a significant risk factor for hypoxic ischemic (HI) encephalopathy in newborns. Mitochondrial wellbeing is critical for cell survival. However, the effect of maternal smoking on mitochondrial damage following HI injury in offspring is not yet known. Female Balb/c mice were exposed to air (SHAM) or cigarette smoke (SE) for 6 weeks prior to mating, during pregnancy and lactation. At postnatal day (P) 10, half of the male pups in each litter underwent left carotid artery occlusion followed by hypoxia (SHAM-HI and SE-HI) and were sacrificed on P45. Proteins were measured using immunoblotting. Mitochondrial oxidative phosphorylation (OXPHOS) complexes III, IV and V were reduced in SE compared to SHAM offspring. OXPHOS complexes I, II and III were increased in SE-HI compared to SE offspring. Mitochondrial fission marker Parkin-8 was reduced but light chain (LC)3A/B-I was increased by maternal SE. HI injury reduced mitophagy markers phosphatase and tensin homolog-induced putative kinase-1, mitochondrial fusion marker Optic atrophy-1, and LC3A/B–I and II protein in the SE offspring. Mitochondrial density in the cerebral cortex was reduced in SHAM-HI and SE-HI compared to SHAM and SE offspring, respectively. HI injury led to an increase in the mitochondrial fission marker while maternal smoking elicited an even higher level in the injured offspring. Our results suggest that maternal SE can worsen mitochondrial damage in offspring following HI injury

Poster 69 – Tuesday 6th December

HYPOXIC ISCHEMIC BRAIN INJURY ACTIVATES INFLAMMATORY AND APOPTOTIC PATHWAYS IN THE NEONATE PIG MODEL

Dr Kirat Chand[1], Ms Kate Goasdoue[1], Ms Stephanie Miller[1], Dr Paul Colditz[1], Dr Tracey Bjorkman[1]

[1]Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland

Perinatal hypoxic-ischemic (HI) brain injury is a common cause of neurodevelopmental outcomes such as cerebral palsy. HI induces activation of inflammatory pathways, which play a significant role in the severity of brain injury. Activation of these mediators also induces apoptotic pathway activity which can lead to further degradation of neuronal structure and function. While the influence of these pathways in progression of HI brain injury in the adult is well studied, there is minimal research into their role in neonatal brain injury following HI. Understanding the impact and time course of these mediators may aid in advancement of treatments to improve neurodevelopmental outcomes of neonates after HI injury. METHODS: Newborn piglets (<24 h after birth) underwent induced hypoxia (n=4) or sham (n=4) for this study. Piglets were euthanized 72 hours after commencement of treatment and brain tissue was collected post-HI. Gene expression of inflammatory and apoptotic markers and morphological changes including microglia (Iba1), astrocytes (GFAP) and degenerating neurons (FJC) were examined. RESULTS: We found a significant upregulation of pro-inflammatory and apoptotic markers in HI animals compared to controls. These changes were associated with alterations in morphological expression of astrocytes and microglia. In addition to this we observed a substantial increase in the number of degenerating neurons in the neonate brain post HI. CONCLUSION: Our results highlight the significant role of inflammatory and apoptotic mediators in progression of HI brain injury in the neonate.

Poster 70 – Monday 5th December

LOSS OF LAMININ-a4 RESULTS IN PRE- AND POSTSYNAPTIC COMPENSATORY MECHANISMS AT THE NEUROMUSCULAR JUNCTION

Dr Kirat Chand[1,2], Ms Kah Meng Lee[1], Dr Nickolas Lavidis[1], Dr Peter Noakes[1,3]

[1]School of Biomedical Sciences, The University of Queensland, [2]Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, [3]Queensland Brain Institute, The University of Queensland

Synaptic basal lamina such as laminin-421 (α4β2γ1), play an integral role in the organisation of the neuromuscular junction (NMJ). Laminins interact with their pre- or postsynaptic receptors to provide stability and ensure precise alignment of the pre- to postsynaptic specialisations. Targeted mutation of the *lama4* gene does not alter formation of active zones (AZs) and junctional folds, though disruptions in the precise alignment of AZs and postsynaptic folds are seen in the NMJs of laminin-α4 knockout mice (*lama4*$^{-/-}$) when compared to wild-type mice (WT). However, *lama4*$^{-/-}$ NMJs display disruptions in the precise alignment of the active zones and postsynaptic folds at the NMJ, although the physiological consequences of this loss have not been previously examined. The present study investigated the differences in neurotransmission during the early development (postnatal day 8) and maturation (postnatal day 18) of the NMJ in *lama4*$^{-/-}$ and WT mice (*n*=6 for each genotype). We observed a significant decrease in miniature end-plate potential (mEPP) frequency, increased amplitude of mEPPs and evoked EPPs, and a decrease in quantal release attributed to fewer active release sites at *lama4*$^{-/-}$ NMJs. Mutant NMJs also displayed higher levels of synaptic depression under high frequency stimulation and altered facilitation, suggesting compromised delivery of synaptic vesicles. Our molecular findings showed altered distribution of synaptic vesicles and the AZ marker Bassoon at the presynaptic terminal. Our results demonstrate a significant role for laminin-a4 in maintaining synaptic efficacy during development of the rodent NMJ.

Poster 71 – Tuesday 6th December

CALRETININ IMMUNOREACTIVE GANGLION CELLS IN THE MARMOSET RETINA.

Miss Ashleigh Chandra[1], Dr Sammy Lee[1,2], Associate Professor Ulrike Grunert[1,2,3]

[1]Department of Ophthalmology and Save Sight Institute, [2]Australian Research Council Centre of Excellence for Integrative Brain Function, University of Sydney Node, [3]School of Medical Sciences, The University of Sydney

Calcium binding proteins such as calretinin are widely used as immunohistochemical markers for a variety of neuronal populations. The aim of this study was to characterize calretinin positive cell types in the ganglion cell layer of the marmoset retina. Marmoset retinas (n=5) were obtained at the end of unrelated electrophysiological experiments and fixed in 4% paraformaldehyde. Retinal quadrants were processed with antibodies to RBPMS, a marker for all ganglion cells, and antibodies to calretinin. Other retinas were pre-labeled with antibodies to calretinin and subsequently intracellularly injected with DiI. Stacks of images were obtained with a confocal microscope. Calretinin immunoreactive cells in the ganglion cell layer comprised ganglion cells and displaced amacrine cells. The ganglion cells made up 18% of the ganglion cell population. A total of 88 DiI injected calretinin positive cells in the ganglion cell layer were analysed. The presence of an axon was used to distinguish ganglion cells from amacrine cells. The ganglion cells were classified according to their dendritic field size, stratification, eccentricity, soma size, and number of dendritic thorns. Using these criteria 38 broad thorny, 28 narrow thorny, 11 parasol ganglion cells and 11 displaced amacrine cells were distinguished. Calretinin immunoreactivity is preferentially expressed by broad and narrow thorny ganglion cell types. We conclude that calretinin may be used as a marker for a subset of wide-field ganglion cells in the marmoset retina.

Poster 72 – Monday 5th December

NEURONAL CONTRIBUTIONS TO MOTION DISCRIMINATION ARE STRONGLY INFLUENCED BY SPIKING RATE, NOT TUNING BANDWIDTH

Mr Tristan Chaplin[1,2,3], Dr Maureen Hagan[1,2,3], Dr Nicolas Price[1,2,3], Professor Marcello Rosa[1,2,3], Dr Leo Lui[1,2,3]

[1]Department of Physiology, Monash University, [2]Neuroscience Biomedicine Discovery Institute, Monash University, [3]Centre of Excellence for Integrated Brain Function, Monash University

The motion sensitivity of neurons in the middle temporal area (MT) of primates is often measured using random-dot stimuli and manipulating the coherence (strength) of the motion signal. The lowest motion coherence in which the firing rate of a neuron reliably distinguishes its preferred direction of motion from the non-preferred is termed the "neurometric" threshold, analogous to the behavioural psychometric threshold. Studies in macaques have shown that MT neurons show a wide range of neurometric thresholds, some approaching the psychometric threshold of the animal. It is unknown if marmoset MT neurons have similar thresholds, and what factors contribute to the variation in thresholds.

We measured the neurometric thresholds of MT single (n=54) and multi-units (n=160) in 9 anesthetised marmosets using both single and multi-electrode arrays. Neurons showed a wide range of thresholds, some as low as 10% coherence, with population median of 55% coherence, similar to reported values in macaques. Maximum firing rate was the strongest predictor of a cell's threshold (r^2=0.41,$p<0.001$). The cell's response to motion in the non-preferred direction was also important (r^2=0.23,$p<0.001$), yet direction-tuning bandwidth showed no correlation with threshold (r^2=0.01,p=0.46). There was no difference in the median neurometric threshold of single electrode recordings, in which the stimuli were optimised for each cell, and array recordings, which used standardised stimuli (Wilcoxon rank-sign,p=0.29). In summary, these results show that the sensitivity of MT neurons is largely determined by the maximum firing rate of the neuron, and suggest that marmosets will be able to perform motion discrimination tasks adequately.

Poster 73 – Tuesday 6th December

INHIBITION OF ENDOCANNABINOID CATABOLIC ENZYME ALLEVIATES NITRERGIC-RELATED NEUROINFLAMMATION FOLLOWING ACUTE STRESS

Hsiao-Jou Cortina Chen[1], Dr Jereme Spiers[1], A/Prof Conrad Sernia[1], Dr Nickolas Lavidis[1]

[1]The University Of Queensland

Immune dyshomeostasis plays a major role in the pathophysiology of stress-related illnesses, with chronic exposure to stress causing neuroinflammation partly through sustained overproduction of free radicals including nitric oxide (NO). We have previously demonstrated that inducible nitric oxide synthase (iNOS) activity and mRNA expression are significantly upregulated in the rat hippocampus following 4 hours of acute restraint stress. Similar to NO, endocannabinoids are synthesised on demand, with preclinical observations suggesting that cannabinoid receptor agonists and endocannabinoid enhancers inhibit nitrergic activity. Specifically, enhancement of endocannabinoids via fatty acid amide hydrolase (FAAH) inhibition with PF-3845 reduced iNOS-expressing microglia following traumatic brain injury. Although cannabinoid modulation of the nitrergic system during pathological conditions has been shown, the acute physiology following a single episode of stress has not been well characterised. The present study examined the effects of systemically injected PF-3845 in the modulation of hippocampal nitrergic and inflammatory-related markers during acute stress. Following vehicle or PF-3845 injections (5 mg/kg; i.p.), male Wistar rats were exposed to 0 (control), 60, 240, or 360 minutes of restraint stress (n=6/group) after which plasma and hippocampus were isolated for further analysis. The results demonstrated that pre-treatment with PF-3845 rapidly reduces stress-induced plasma corticosterone release. This was accompanied by a reduction in the proinflammatory response including iNOS, TNFα converting enzyme, IL-1β, IL-6, and cyclooxygenase-2 mRNA levels in the hippocampus. Furthermore, transcriptional potential of NF-κB was inhibited by PF-3845 suggesting that enhanced endocannabinoid levels in the hippocampus have an overall anti-nitrosative and immunosuppressive effect following acute stress exposure.

Poster 74 – Monday 5th December

THE FINE-SCALE STRUCTURE OF SPONTANEOUS SYNAPTIC ACTIVITY IN THE DEVELOPING MOUSE VISUAL CORTEX

Dr Juliette Cheyne[1], Dr Nawal Zabouri[2], Dr Christian Lohmann[2]

[1]University Of Auckland, [2]Netherlands Institute for Neuroscience

Neurons in the developing brain are spontaneously active before the onset of sensation. This spontaneous activity is crucial for development of the specific neuronal networks that are required for normal brain function. We combined dendritic calcium imaging with whole cell electrophysiology *in vivo* to map synaptic inputs on the dendritic tree during spontaneous network events. Previously we found that synaptic inputs are already organized with subcellular precision before eye opening, such that neighboring synapses are functionally clustered. We also uncovered an "out-of-sync, loose-your-link" local plasticity rule that is driven by spontaneous activity. We aimed to further define synaptic clusters functionally and to reveal their presynaptic partners. We find that while clusters vary greatly in size, the same sets of synapses can be active repeatedly. We are beginning to reveal the presynaptic partners to these synapses using whole brain immunohistochemistry and brain clearing techniques. We find that synaptic clusters can include synapses of mixed origin, as both thalamo-cortical synapses and cortical-cortical synapses are present within them. These data increase our understanding of the fundamental logic of the clustered synaptic organization that is established early in development.

Poster 75 – Tuesday 6th December

AAV-MEDIATED EXPRESSION OF BDNF AND CRMP2 PROMOTES DIFFERENTIAL LONG TERM NEUROPROTECTION OF ADULT RAT RETINAL GANGLION SOMATA AND AXONS FOLLOWING PARTIAL OPTIC NERVE INJURY

Ms Wissam Chiha[1,2,3], Ms Carole Bartlett[1,3], Dr Steven Petratos[4], Dr Melinda Fitzgerald[1,3], Professor Alan Harvey[2,5]

[1]Experimental and Regenerative Neurosciences, The University of Western Australia, [2]School of Anatomy, Physiology and Human Biology, The University of Western Australia, [3]School of Animal Biology, The University of Western Australia, [4]Monash University, Melbourne, Australia , [5]Western Australian Neuroscience Research Institute

Neurotrauma leads to immediate degeneration of impacted neural tissue and secondary degeneration of initially intact adjacent areas. Secondary degeneration can be modelled by partial transection (PT) of the dorsal rat optic nerve (ON). Retinal ganglion cells (RGCs) in dorsal retina project their axons to dorsal ON, which are axotomised by the primary injury; RGCs in central retina are affected by both primary and secondary degeneration. Ventrally located RGCs are not directly damaged but remain vulnerable to secondary degeneration. The effects of intravitreally administered bi-*cis*tronic adeno-associated viral-vectors (AAV-2) encoding BDNF and/or non-phosphorylatable collapsing response mediator protein-2 (CRMP2) with green fluorescent protein (GFP) on RGC survival and axonal integrity were assessed following PT injury. Three months after injury, RGC numbers declined by over 60% in AAV-GFP controls in dorsal and central retina and by 56% in ventral retina, relative to uninjured controls ($p \leq 0.05$). The number of RGCs in dorsal retina was not significantly enhanced by growth factor treatment, while in central retina, all treatments restored RGC numbers to uninjured levels. In ventral retina, RGCs were significantly protected only after treatment with AAV vectors encoding BDNF ($p \leq 0.01$). *S*ignificant decreases in βIII-tubulin immunoreactivity were observed in dorsal and ventral ON following PT. AAV mediated expression of CRMP2, with or without BDNF, significantly upregulated βIII-tubulin immunoreactivity in ON, relative to AAV-GFP following injury ($p \leq 0.05$). Overall, the data suggest that the maintenance of axonal

integrity and cell viability during primary and secondary injury events are dissociable, and may be regulated and influenced by different cellular mechanisms

Poster 76 – Monday 5th December

CHARACTERISATION OF RXFP3+ NEURONS IN MOUSE BED NUCLEUS OF THE STRIA TERMINALIS: NEUROCHEMICAL PHENOTYPING

Ch'ng SS, Cornish L, Smith CM, Brown RM, Gundlach AL, Lawrence AJ.

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

The bed nucleus of the stria terminalis (BNST) is a stress-sensitive region with strong projections to the ventral tegmental area (VTA) that are implicated in reward-seeking. The BNST is highly heterogeneous with neurochemically distinct neuronal subpopulations, and contains cells expressing relaxin family peptide 3 receptor (RXFP3), the cognate receptor for neuropeptide relaxin-3. However, the neurochemical phenotype of RXFP3+ cells has yet to be characterised. In RXFP3-Cre mouse BNST there are at least two distinct classes of RXFP3+ neurons: a presumed excitatory population expressing vesicular glutamate transporter 2 (vGluT2) and a GABAergic population, the latter comprising ~25% of RXFP3+ cells. Furthermore, ~21% of RXFP3+ neurons co-express calbindin, a cortical inhibitory marker. The retrograde tracer cholera toxin β (80-100nL) was injected intra-VTA to ascertain if BNST RXFP3+ neurons are VTA-projecting, but BNST RXFP3+ neurons do not appear to project directly to the VTA. To assess the connectivity of RXFP3+ neurons, RXFP3-Cre mice were injected intra-BNST with AAV-hSyn-mDIO-GFP anterograde viral tracer (400nL) to determine BNST RXFP3+ efferents. Additionally, to examine if BNST RXFP3+ cells are inherently stress-sensitive, RXFP3-Cre mice were subjected to an acute stressor (6 minute swim stress), anaesthetised (pentobarbitone, 80mg/kg i.p.) and perfused 90 minutes later. Around 13% of RXFP3+ neurons in stressed animals expressed Fos immunoreactivity, suggesting a subset of BNST RXFP3+ cells are part of an integrated network that is sensitive to acute stressors. Overall, these studies will help to delineate the phenotype and connectivity of the BNST RXFP3+ system and its involvement in stress reactivity

Poster 77 – Tuesday 6th December

ALTENUSIN REDUCES BOTH TAU AGGREGATION IN VITRO AND TAU PHOSPHORYLATION IN CELL CULTURE MODELS

Dr Sook Wern Chua[1,2], Dr Alberto Cornejo[3], Prof Michael Kassiou[4], Prof Lars Ittner[1,2]

[1]UNSW, [2]NeuRA, [3]University Andrés Bello, [4]University of Sydney

The neuropathology behind Alzheimer's disease is linked to protein deposition in extracellular amyloid-β plaques and intracellular neurofibrillary tangles (NFTs) that consists of the microtubule-associated protein tau. Paramount to the formation of NFTs is hyperphosphorylation of tau that results in the conversion of soluble tau into insoluble filaments and aggregates. In this study, altenusin, a bioactive polyphenolic compound isolated from *Penicillium*, was assessed for its potential to inhibit tau aggregation formation. The results show that altenusin is able to inhibit aggregation of tau proteins into paired helical filaments (PHFs) *in vitro*. This was associated with stabilisation of tau dimers and other oligomers into globular structures as revealed by atomic force microscopy. Cell culture experiments showed that altenusin reduced levels of tau phosphorylation in P301L mutant tau-expressing cells. Furthermore, altenusin reverses the neuritic tau pathology in a dose dependent manner, as induced by incubation of primary cultured neurons with tau fibrils. However, *in vivo* treatment of P301S mutant tau transgenic mice did not improve neuropathology and functional deficits. Taken together, altenusin prevents tau fibrillisation *in vitro* and induced tau pathology in neurons, but does not show therapeutic efficacy in an initial trial in a mouse model of Alzheimer's disease.

Poster 78 – Monday 5th December

CHARACTERISATION OF RXFP3+ NEURONS IN MOUSE BED NUCLEUS OF THE STRIA TERMINALIS: ELECTROPHYSIOLOGY

Ms Jingjing Fu[1,2], Dr Stuart McDougall[1], Dr Robyn Brown[1], Prof Andrew Lawrence[1]

[1]The Florey Institute of Neuroscience and Mental Health, [2]School of Pharmaceutical Sciences, Tsinghua University

The relaxin-3/relaxin family peptide receptor 3 (RXFP3) system modulates stress responses and reward seeking behaviour. RXFP3 is expressed within the bed nucleus of the stria terminalis (BNST), a stress-sensitive region. The electrophysiological properties of RXFP3+ neurons and their potential interaction with other neuropeptides such as corticotropin releasing factor (CRF) within the BNST are not known. We conducted whole cell recordings from RXFP3+ neurons in brain slices taken from adult RXFP3-Cre mice (n=16). Mice were anaesthetised (5% isoflurane), decapitated and 250 um coronal slices through the BNST were obtained. Based on Hammack's characterization (Hammack, Mania, & Rainnie, 2007), all of the currently recorded RXFP3+ neurons were classified Type II BNST neurons which constitute 55% of the BNST population. To discover the pharmacological effects of CRF on these RXFP3+ neurons, we bath applied CRF (100-300 nM) onto brain slices during gap free voltage clamp recordings (n=9). CRF increased GABA release onto a subset of RXFP3+ neurons. Future studies will further examine of bath application of a RXFP3 agonist and other peptides on RXFP3-Cre BNST slices. In addition, similar recordings will be conducted after chronic swim-stress to determine if these are implicated in stress response circuitry. Overall, these experiments will help to elucidate the electrophysiological properties of the BNST cells that express RXFP3, their sensitivity to stress and their interaction with well known stress neuropeptides.

Poster 79 – Tuesday 6th December

TRANSCRIPTOME PROFILING OF FEZF2-EXPRESSING INTRATELENCEPHALIC-PROJECTION NEURONS IN THE MATURE MOUSE MOTOR CORTEX

Alison J Clare[1,3,4], Dr Robert C Day[1,3], Associate Professor Ruth M Empson[2,4], Dr Stephanie M Hughes[1,3,4]

[1]Department of Biochemistry, University of Otago, [2]Department of Physiology, University of Otago, [3]Genetics Otago, University of Otago, [4]Brain Health Research Centre, University of Otago

The mature cortex hosts hugely diverse populations of pyramidal neurons. Characterising this complexity is essential to our understanding of the healthy cortex. We recently demonstrated a distinct identity for *Fezf2*-positive intratelencephalic-projection neurons (IT-PNs) from layer 5 of the motor cortex. When compared to *Fezf2*-negative IT-PNs, within the same layer, these neurons displayed a unique apical dendritic tuft and electrophysiological phenotype. Identifying the molecular profiles that underpin these properties is crucial to understanding the role and maintenance of *Fezf2+* IT-PN types. We developed a method, using a *Fezf2*-GFP reporter mouse, where *Fezf2+* and *Fezf2-* IT-PNs were identified and collected by *in vivo* retrograde labelling and fluorescence activated cell sorting. Applying a novel low-input RNA-sequencing method, we have generated transcriptome profiles and identified a set of 197 genes with significant changes in expression between *Fezf2+* and *Fezf2-* IT-PNs (n = 5, false discovery rate < 0.1). Further bioinformatics analysis of genes over-represented in *Fezf2+* IT-PNs identified significant enrichment of the calcium-binding EF-hand domain ($p < 0.05$). This finding alludes to a greater importance for calcium handling in the *Fezf2+* IT-PNs. In particular, calcium signalling is important for synaptic plasticity and dendritic spine remodelling and could be critical to the distinct dendritic morphology observed in *Fezf2+* IT-PNs. Our dataset is the first to identify the molecular profiles of *Fezf2+* IT-PNs. Future work will investigate the importance of target genes identified in maintaining *Fezf2+* IT-PNs in the mature brain. This will be essential for understanding the function of these IT-PNs in health and disease.

Poster 80 – Monday 5th December

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION AFFECTS GLIAL SCARRING IN A MOUSE MODEL OF BRAIN INJURY.

Mr Darren Clarke[1], A/Prof Jennifer Rodger[1], E/Prof Alan Harvey[2], Mrs Marissa Penrose[1], Dr Kristyn Bates[1]

[1]School of Animal Biology, University Of Western Australia, [2]School of Anatomy, Physiology and Human Biology, University of Western Australia

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive form of brain stimulation that can modulate neuronal activity depending on the frequency of pulses administered. However the effects on glial cells are largely unknown. Astrocyte and microglial reactivity is a characteristic response to brain injury that can have both beneficial and detrimental impacts on functional recovery. Additionally, age and sex influence glial cell response to injury, creating further complications in the development of treatments. In this study, we examined the effect of rTMS on the glial response to brain injury, and how age and sex may influence this effect. We applied focal low intensity rTMS at a range of frequencies for 14 days, following unilateral penetrating cortical stab injury in 3 month and 18 month female and male wild-type C57BL6/J mice. The applied frequencies were 1Hz, a complex 6-10Hz, and sham ipsilaterally, and 1Hz contralaterally. The spread and local response of reactive astrocytes and microglia, and proteoglycan intensity and area expression surrounding the injury was quantified by immunohistochemical staining of serial brain sections. rTMS had significant age, sex, and frequency specific effects on the local response of glia following injury. Specifically, rTMS increased the density of astrocytes and microglia surrounding the lesion in male mice, but had the opposite effect in females. The data thus lend some support to the therapeutic potential of rTMS, but further investigation into rTMS modulation of glial scarring is needed to define parameters for tailoring rTMS treatment to the individual.

Poster 81 – Tuesday 6th December

FUNCTIONAL ANALYSIS OF THE BDNF VAL66MET POLYMORPHISM IN DONOR-SPECIFIC NEURONS

Dr Anthony Cook[1], Ms Sree Koilkandadai[1], Dr Duncan Crombie[2], Dr David Ward[1], Dr Herlena Liang[2], Maciej Daniszewsi[2], Tejal Kulkani[2], Fan Li[2,3], Justin Dittmann[1], Dr Raymond Wong[2], Dr Anna King[1], Assoc Prof Alice Pébay[2], Assoc Prof Alex Hewitt[2,3,4], Professor James Vickers[1]

[1]Wicking Dementia Research And Education Centre, University Of Tasmania, [2]Centre for Eye Research Australia, [3]Menzies Institute for Medical Research, [4]School of Medicine, University of Tasmania

The BDNF Val66Met polymorphism moderates the rate of memory decline in preclinical Alzheimer's disease. Similarly, a major finding from the Tasmanian Healthy Brain Project has been that BDNF Met carriers with low baseline cognitive reserve exhibited a 36-month declining trajectory in executive function that was not observed in BDNF Met carriers with high baseline cognitive reserve ($p = 0.007$). This finding implicates the BDNF Met allele in having a detrimental effect on ageing-related cognitive change and, potentially risk of dementia. However, how the BDNF Val66Met polymorphism affects neuron biology is only partially understood. To identify measureable neuronal behaviours and molecular profiles associated with BDNF genotype, we have established over 100 iPS cell lines corresponding to participants within the Tasmanian Healthy Brain Project. Episomal vectors containing OCT4, SOX2, KLF4, L-MYC, LIN28, and shRNA against p53 were nucleofected into fibroblasts, iPS cells selected using live immunostaining of the pluripotent marker TRA-1-60, and assessed for pluripotency by embryoid body formation. In parallel, we are utilising CRISPR/Cas technology to generate isogenic pairs of pluripotent stem cell lines homozygous for either BDNF allele. We are also implementing direct fibroblast-to-neuron conversion protocols using defined combinations

of cell-permeable small molecules in order to measure BDNF genotype-specific effects in neurons with preserved age-related epigenetic signatures, and have generated Tuj1 positive cells with morphology indicative of neurons. Combined, these complementary approaches enable a thorough investigation of the BDNF Val66Met polymorphism, and may provide significant advances in understanding the mechanism by which the BDNF Met allele increases dementia risk

Poster 82 – Monday 5th December

THE EFFECT OF ALCOHOL EXPOSURE FOLLOWING REPEATED CONCUSSION

Dr Frances Corrigan[1], Jessica Sharkey[1], Emma Thornton[1], Lyndsey Collins-Praino[1]

[1]School of Medicine, University Of Adelaide

A history of repeated concussion has been linked to the later development of the neurodegenerative disease, chronic traumatic encephalopathy (CTE), which is characterised by the accumulation of hyperphosphorylated tau. How repeated concussion promotes neurodegeneration remains poorly understood and external factors may play a role in disease development. Of particular interest is the role of alcohol consumption, with up to a quarter of people consuming alcohol following a concussion, which enhances neuroinflammation and worsens outcome. However the effect of alcohol following repeated concussion is unknown. To investigate male Sprague-Dawley rats were subject to three concussions 5 days apart using the diffuse impact-acceleration model to generate ~100G. Sham animals underwent surgery only. 5 days following last injury alcohol (3.5mg/kg or 5mg/kg) or dextrose via oral gavage was administered, with rats perfuse-fixed 24hrs later for examination of levels of inflammation and phosphorylated tau (ptau). A significant increase in ptau within the cortex was only seen in repeated concussion animals administered 5mg/kg of alcohol ($p<0.05$ compared to vehicle controls), with no effect of the lower dose of alcohol. Intriguingly enhanced inflammation, as assessed by the number of GFAP positive cells was only seen in the repeated concussion animals treated with 3.5mg/kg of alcohol, with the higher dose (5mg/kg) appearing to reduce the number of GFAP positive cells. No effects of alcohol were seen in sham animals. Alcohol appears to have dose-dependent and contradictory effects on inflammation and tau phosphorylation following repeated concussion, with further research needed to determine whether this has functional consequences.

Poster 83 – Tuesday 6th December

TIME-DEPENDENT EFFECTS OF TOLL-LIKE RECEPTOR 4 ACTIVATION FOLLOWING REPEATED MILD TRAUMATIC BRAIN INJURY

Dr Frances Corrigan[1], Lyndsey Collins-Praino[1], Alina Arulsamy[1], Robert Vink[2]

[1]School of Medicine, University Of Adelaide, [2]University of South Australia, Division of Health Sciences, Adelaide

Repeated mild traumatic brain injury (rmTBI), is associated with development of the neurodegenerative disorder, chronic traumatic encephalopathy (CTE), characterised by the accumulation of hyperphosphorylated tau. The link between rmTBI and later CTE is poorly understood and external factors may play a role. Of interest is the effect of activation of the innate immune receptor, toll-like receptor 4 (TLR4). To investigate male Sprague-Dawley rats were administered three mTBIs 5 days apart using the diffuse impact-acceleration model to generate ~100G. Sham animals underwent surgery only. At 1 or 5 days following last injury rats were given the TLR4 agonist, lipopolysaccharide (LPS, 0.1mg/kg), or saline. LPS administration regardless of time-point acutely increased tau phosphorylation post-rmTBI. However, LPS given at 1D enhanced neuroinflammation increasing microglial reactivity ($p<0.05$), whereas LPS at 5D post-injury impaired the inflammatory response with a significant decrease in CD68+ve cells ($p<0.01$). This had long-term detrimental consequences with rats given LPS at 5D post-injury exhibiting impaired cognition on the Barnes Maze and increased depressive-like behaviour on the Forced Swim Test 3 months post-injury ($p<0.05$), with a reduction in synaptic density ($p<0.05$). Conversely, augmenting inflammation with LPS at 1D post-injury reduced depressive-like behaviour and improved cognition ($p<0.05$), with these animals no different to shams. By 3 months post-injury enhanced tau phosphorylation was only seen in vehicle and 5D LPS treated rmTBI rats. Thus, activation of TLR4 immediately after rmTBI is potentially protective, through increased microglial reactivity, whereas delayed activation of TLR4 may be detrimental due to a dampening of the microglial response.

Poster 84 – Monday 5th December

THE IMPACT OF 1,25-DIHYDROXYVITAMIN D ON L-TYPE VOLTAGE-GATED CALCIUM CHANNEL TRAFFICKING IN RAT CORTICAL NEURONS

Dr. Xiaoying Cui[1], Dr. Men Chee Tan[1], Dr. Victor Anggono[1], Dr. Helen Gooch[1], Dr. Thomas Burne[1,2], Dr. Darryl Eyles[1,2], Dr. John McGrath[1,2]

[1]Queensland Brain Institute, The University Of Queensland, [2]Queensland Centre for Mental Health Research

Developmental vitamin D deficiency is an established risk factor for schizophrenia. The impact of $1,25(OH)_2D_3$ (the active form of vitamin D) on brain development via its classical genomic pathways is well documented, however, less is known about the non-genomic functions of this vitamin. One non-genomic function of 1, $25(OH)_2D_3$ is to rapidly enhance calcium influx via L-type voltage-gated calcium channels (L-VGCC) and this has been demonstrated in bone and muscle. Considering the accumulation of evidence linking common genetic variants in L-VGCC with schizophrenia, we investigated the impact of 1, $25(OH)_2D_3$ on L-VGCC activity in rat cortical neurons. Our results showed that 10 minutes of 1, $25(OH)_2D_3$ treatment significantly reduced surface levels of the pore-forming unit of L-VGCC(subunit A1C) without altering its total levels in cultured cortical neurons. Inhibition of the activation of CaMKII, PKA, PKC and PI3K blocked the L-VGCC-A1C

internalisation. Blocking endocytosis or the use of nifedipine also diminished the internalization of L-VGCC-A1C, indicating this process might be L-VGCC-dependent channel endocytosis. It is known that the number of L-VGCCs at the membrane tightly controls the magnitude and kinetics of calcium entry into cells. Alterations in the intracellular calcium concentration could influence a wide range of neuronal functions, such as neuronal differentiation, maturation, dendritic growth and synapses formation. Further experiments are ongoing to investigate whether $1,25(OH)_2D_3$ induced L-VGCC internalization is causal for the other actions of this vitamin/hormone.

Poster 85 – Tuesday 6th December

NON-INVASIVE REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION INCREASES NEWBORN OLIGODENDROCYTE ADDITION IN THE MOUSE CORTEX

Dr Carlie Cullen[1], Mr Alexander Tang[2], Miss Megan O'Rourke[1], Dr Matteo Senesi[1], Dr Jennifer Rodger[2], Dr Kaylene Young[1]

[1]Menzies Institute for Medical Research, University Of Tasmania, [2]Experimental and Regenerative Neurosciences, School of Animal Biology, University of Western Australia

Increased neuronal activity has been shown to increase oligodendrogenesis and myelination in adulthood. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that uses magnetic pulses to induce electrical currents in the underlying nervous tissue. We hypothesised that rTMS would promote cortical oligodendrocyte generation. To investigate this, adult (P90) *Pdgfrα-CreER^{T2} :: Rosa26-YFP* transgenic mice received Tamoxifen to fluorescently label oligodendrocyte progenitor cells (OPCs) and their progeny. From P97 mice received 3 minutes of sub-threshold, non-invasive intermittent theta burst rTMS (120mT coil) or sham stimulation for 14 consecutive days, as well as EdU via their drinking water. Mice were perfused after 1, 3, 7, 10 and 14 days of treatment, and up to 14 days after the cessation of treatment (P90+28 days). Coronal brain sections (30µm) were collected and used to determine the proportion of OPCs that had divided ($PDGFR\alpha^+$ EdU^+ / Total $PDGFR\alpha^+$), as well as the number of oligodendrocytes (YFP^+ $OLIG2^+$ PDGFRA-negative) generated. We found that rTMS did not alter OPC proliferation at any stage examined ($P>0.05$). Similarly, the number of new oligodendrocytes added to the corpus callosum was unaffected by treatment ($P>0.05$). However, mice receiving 14 days of rTMS had approximately twice as many new oligodendrocytes added to targeted cortical regions relative to sham-treated mice (M1: $P=0.03$, Sham 21.18 ± 0.8, rTMS 48.97 ± 9.1; V2: $P=0.02$, Sham 20.67 ± 2.2, rTMS 46.16 ± 6.4 OL's/mm^2). These data suggest that rTMS can increase adult oligodendrogenesis in the healthy nervous system.

Poster 86 – Monday 5th December

AGEING AND THE BLOOD-CNS BARRIERS

Mr Mitchell Cummins[1,2], Dr Doug Smith[1,2]

[1]School of Biomedical Sciences and Pharmacy, Priority Research Centre for Brain and Mental Health Research, The University of Newcastle, [2]Hunter Medical Research Institute

The blood-brain (BBB) and blood-spinal cord (BSCB) barriers protect the neural environment by regulating ion, molecule and cell movement between the blood and CNS parenchyma. Ageing is associated with decreased CNS function, and increased barrier permeability may contribute to this. Until now, a molecular investigation of the effects of ageing on the BBB and BSCB has not been reported. We hypothesised that ageing would be associated with altered barrier gene expression leading to barrier property changes. We used laser microdissection (LM) and qPCR to determine the effects of ageing on barrier cell gene expression. Brain microvessels containing endothelial cells and pericytes were microdissected from frontal cortex and cervical spinal cord grey matter sections from young and old (n=3/group) C57Bl6 mice. Cell specific genes were also analysed in homogenates. LM sample purity was analysed. Claudin-5 (Cldn-5) and immunoglobulin superfamily member 5 (Igsf5) were significantly down-regulated ($p<0.05$, Mann-Whitney U Test) in the BBB of old animals. There was a non-significant trend for down-regulation of occludin (Ocln). In the BSCB, collagen-IVA1 and Igsf5 were down-regulated ($p<0.05$). Trends towards down-regulation were observed for Cldn5, Gja1, Ocln and platelet-derived growth factor β. These data suggest decreased expression of barrier proteins may underpin increased barrier permeability with age. These findings are likely relevant to age-related CNS pathologies, and to extending health-span.

Poster 87 – Tuesday 6th December

DISEASE-ASSOCIATED TAU IMPAIRS MITOPHAGY BY INTERACTING WITH PARKIN AND PREVENTING ITS TRANSLOCATION TO DAMAGED MITOCHONDRIA

Nadia Cummins[1], Dr J Bertran-Gonzalez[1], Prof Jürgen Götz[1]

[1]Queensland Brain Institute, University Of Queensland

A key feature of Alzheimer's disease (AD) is an accumulation of dysfunctional mitochondria, with both Tau protein and amyloid-β, the two central players in AD, impairing diverse aspects of mitochondrial function. Damaged mitochondria can be degraded via autophagic pathways (termed "mitophagy"), which involves translocation of the ubiquitin ligase Parkin from the cytosol to mitochondria, and initiation of the autophagic machinery. Following recent reports describing altered mitophagy in AD, we explored how disease-relevant forms of Tau affect steps of the mitophagy pathway. We found that both wild-type and mutant fronto-temporal dementia-associated P301L-Tau

inhibited Parkin translocation to mitochondria following CCCP-induced depolarisation of mitochondria in neuroblastoma (N2a) cells. This was despite unaltered mitochondrial membrane potential between control and Tau-expressing cells, indicating that the effects of Tau on Parkin translocation occurred downstream of depolarisation.

Interestingly, proximity ligation assays (PLAs) revealed Tau-Parkin interactions at multiple domains of Tau. These interactions were absent for mitochondrial Parkin, suggesting that Tau can trap Parkin in the cytosol and therefore prevent its translocation to mitochondria. In support of this, truncated Tau lacking a microtubule-binding domain, which is therefore mostly cytosolic, was also able to inhibit Parkin translocation.

Finally, in brains of aged P301L-Tau transgenic pR5 mice, which have reported mitochondrial dysfunction, Parkin levels were unchanged in both cytosolic and mitochondrial fractions, suggesting a lack of Parkin-mediated mitophagy in this tauopathy model. Our findings imply that pathological forms of tau may prevent cells from clearing damaged mitochondria effectively, therefore providing a new dimension to tau-mediated toxicity

Poster 88 – Monday 5th December

BDNF SIGNALS TO TRKB RECEPTORS EXPRESSED BY NEURONS TO REGULATE OLIGODENDROCYTE POPULATION AND MYELINATION

Fatemeh Daemi, Rhiannon J Wood, David Gonsalvez, Jessica Fletcher, Simon S Murray and Junhua Xiao

The Department of Anatomy and Neuroscience, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, Victoria, 3010

Background Brain-derived neurotrophin factor (BDNF) has been implicated in controlling central nervous system (CNS) myelination via its tropomyosin related kinase (Trk) B receptors, however the precise cellular and molecular mechanisms by which BDNF/TrkB signalling regulates CNS myelination remains unclear. We have previously identified that BDNF exerts specific influence upon myelin wrapping via activating TrkB receptors expressed by oligodendrocytes. Here we investigated the influence that BDNF/TrkB signalling in neurons exerts upon oligodendroglial cells and myelination during CNS development.
Results We have generated a neuronal-specific TrkB conditional knockout mouse ($TrkB^{flfl}$NFLCre, TrKB cKO) and assessed oligodendroglial populations and myelin formation in key regions of both white matter (spinal cord white matter tracts and corpus callosum) and grey matter (cerebral cortex) at three developmental time points (P6, P14 and P70). We found that TrkB cKO mice displayed fewer oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes in both white and grey matter regions at all time points compared to littermate controls (n=2-3 mice/genotype/time point) which is significant at P6 and P14. The ablation of TrkB expression in neurons has also lead to a change in the number of pre-mature oligodendrocytes in these CNS regions during development. We have also assessed ultrastructure of myelinated axons in lumbar spinal cord white matter tracts and corpus callosum via electron microscopy analysis. Our data showed that TrkB cKO mice had fewer myelinated axons compared to control mice during early postnatal development. Importantly, there are more axons with non compact myelin during CNS development following neuronal TrkB deletion (n=2-3 mice/genotype/timepoint).
Conclusions Together, our data demonstrate that BDNF signals to neuronal TrkB receptors to regulate oligodendroglial lineage progression and the initiation of myelination during CNS development, suggesting a novel role that neuronal signals play in regulating oligodendroglial function.

Poster 89 – Tuesday 6th December

FREQUENCY OF CNKSR2 MUTATION IN X-LINKED EPILEPSY-APHASIA SYNDROME

Mr John A. Damiano[1], Miss Rosemary Burgess[1], Dr. Sara Kivity[2], Dr. Tally Lerman-Sagie[3], Professor Ingrid E. Scheffer[1], Professor Samuel F. Berkovic[1], Dr. Michael S. Hildebrand[1]

[1]University Of Melbourne, [2]Department of Pediatric Neurology and Epilepsy Center, Schneider Children's Medical Center of Israel, [3]Epilepsy Clinic, Pediatric Neurology Unit, Wolfson Medical Center

Objective: Synaptic proteins are critical to neuronal function in the brain and their deficiency can lead to seizures and cognitive impairments. CNKSR2 is a synaptic protein involved in Ras signaling-mediated neuronal proliferation, migration and differentiation. Mutations in the X-linked gene *CNKSR2* have been described in patients with seizures and language, intellectual and attention deficits.
Methods: In this study we sequenced 123 patients with phenotypes within the epilepsy-aphasia spectrum (EAS) and possible X-linked inheritance to determine the frequency of *CNKSR2* mutation within this complex spectrum of disorders.
Findings: We detected a novel nonsense mutation (c.2314 C>T; p.Arg712*) in one Ashkenazi Jewish family, the male proband of which had epileptic encephalopathy with continuous spike-waves in sleep (ECSWS). His affected brother also had ECSWS with a less severe phenotype, while their sister had childhood epilepsy with centro-temporal spikes and a mild learning disability. This mutation segregated in the three affected siblings in an X-linked dominant manner, inherited from their mother who had febrile seizures.
Conclusions: Although the frequency of point mutation is low, *CNKSR2* sequencing should be considered in families with suspected X-linked EAS because of the self-limiting nature of the phenotype and specific genetic counseling implications.

Poster 90 – Monday 5th December

ALTERED CARDIAC FUNCTION AND STRUCTURE IN AN ACQUIRED ANIMAL MODEL OF CHRONIC TEMPORAL LOBE EPILEPSY

Mr Luke D'arcy[1], Dr Claire Curl[2], Mrs Emma Braine[1], Ms Hanneke Raaijmakers[2], Professor Colin Royse[3], Professor Lea Delbridge[2], Professor Terence O'Brien[1], Dr Kim Powell[1]

[1]Department of Medicine, University Of Melbourne, [2]Department of Physiology, University Of Melbourne, [3]Department of Surgery, University Of Melbourne

Background: Cardiac dysfunction is common in patients with chronic epilepsy. This may develop into cardiac arrhythmias leading to an increased risk of Sudden Unexplained Death in EPilepsy (SUDEP). Previous research investigating the relationship between cardiac dysfunction and epilepsy is limited; therefore, this study investigated cardiac structural and functional alterations during epileptogenesis, and in the chronic epileptic period, in the post-SE model of acquired epilepsy.
Methods: 10 week old Wistar rats were treated with kainic acid to induced status epilepticus (SE) for 4 hours (n=10), while control rats received saline (n=8). Echocardiography was acquired pre-SE, 2 and 10 weeks post-SE. Rats underwent implantation of EEG recording electrodes and were monitored for 2 weeks for seizure frequency and severity. Following euthanasia hearts were excised for histological analysis.
Results: EEG analysis showed seizure frequency ranging from 0.08 to 10.7 seizures/day. Echocardiographic analysis 10 weeks post-SE showed reduced chamber dimensions during diastole ($p<0.05$) and altered diastolic function with decreased mitral valve deceleration time ($p<0.05$) and myocardial velocity changes ($p<0.05$) in epileptic rats compared to controls. Myocardial fibrosis deposition was significantly increased in post-SE rats ($p<0.001$) and was positively correlated with seizure frequency ($r^2=0.975$).
Conclusion: Cardiac dysfunction in post-SE rats is progressive during epileptogenesis and displays structural and functional alterations at the chronic epilepsy time point that was correlated with seizure frequency. This indicates that chronic epilepsy results in secondary myocardial changes and cardiac dysfunction that may increase the risk of cardiac arrhythmias, and potentially have pathological importance in patients with chronic drug resistant epilepsy

Poster 91 – Tuesday 6th December

EFFECTS OF CENTRAL (ICV) ADMINISTRATION OF RELAXIN-3 OR AN RXFP3-SELECTIVE AGONIST ON FOOD INTAKE AND CIRCULATING HORMONES IN ADULT MALE RATS

B.Sc. Camila de Ávila[1], Ph.D Sandrine Chometton[1], Research assistant Geneviève Guevremont[1], Ph.D Juliane Calvez[1], Ph.D Christophe Lenglos[1], Ph.D Andrew L. Gundlach[2], Ph.D Elena Timofeeva[1]

[1]Université Laval, [2]The Florey Institute of Neuroscience and Mental Health

Relaxin-3 is a well-conserved neuropeptide member of the relaxin-peptide family, produced by GABA neurons in specific brain areas, including *nucleus incertus*, and is implicated in motivated behaviours and stress responses. Relaxin-3 preferentially activates the $G_{i/o}$-protein-coupled receptor, RXFP3, but can activate RXFP1, the cognate G_s-protein-coupled receptor for relaxin, both *in vitro* and *in vivo*. In contrast, the truncated peptide, RXFP3-A2 (A2), is a highly-selective RXFP3 agonist *in vitro*/*in vivo*. In this study we assessed comparative responses to central administration of relaxin-3 and A2 on feeding and circulating hormone levels in adult male rats. Acute intracerebroventricular (icv) injection of relaxin-3 and A2 (1.1 nmol, n=12/group) increased food intake (30-120 min), with a more rapid response to A2 (relaxin-3, $p\leq0.0054$ vs A2, $p\leq0.0001$ after 30 min). Relaxin-3, but not A2 increased water intake, and increased plasma testosterone and corticosterone ($p\leq 0.0001$, 60 min post-injection) suggesting a role for RXFP1, not RXFP3. In addition, *in situ* hybridization studies revealed different patterns of c-*fos* mRNA (reflecting differential neuronal activity) in the paraventricular hypothalamic nucleus (PVN), supraoptic nucleus (SON) and medial preoptic area (MPA) of relaxin-3- vs A2-treated and vehicle-treated rats. In contrast, both icv relaxin-3 and A2 increased c-*fos* mRNA levels in the lateral hypothalamic area (LHA). These results further illustrate the differential *pharmacological* effects of RXFP1 and RXFP3 activation on key hypothalamic networks, including those neural circuits regulating feeding and the hypothalamus-pituitary-gonadal (HPG) axis. Ongoing studies aim to determine which of these effects represent *physiological* actions of relaxin-3 signalling at RXFP1 and/or RXFP3.

Poster 92 – Monday 5th December

COMBATTING THE HIPPOCAMAPAL EFFECTS OF EARLY LIFE OVERFEEDING BY MINOCYCLINE

Miss Simone De Luca[1], Mrs Ilvana Ziko[1], Mr Alita Soch[1], Ms Luba Sominsky[1], Ms Sarah Spencer[1]

[1]RMIT University

The early life nutritional environment can program microglial proliferation within the hypothalamus, with a predisposition to a central pro-inflammatory profile long-term contributing to overactive immune responses. This microgliosis extends into memory-associated brain regions resulting in spatial memory deficits. We hypothesize that long-term microgliosis caused by over nutrition during development will result in a lasting central pro-inflammatory profile in the hippocampus. To test this idea, we manipulated the litter sizes of rats ensuring that the pups were suckled in litters of 4 (neonatally overfed) or 12 (control). Concomitantly, we also gave the rats daily injections of anti-inflammatory antibiotic, minocycline or vehicle, during the developmental period, to normalize the acute inflammatory response in neonatally overfed rats. During adulthood we then observed the effects of early life overfeeding on immune responses to lipopolysaccharide and whether these responses were attenuated with minocycline. The hippocampal responses were examined via detection of microgliosis and changes in neuronal profiles. Neonatally overfed rats have evidence of microgliosis in the CA1, CA3 and

dentate gyrus regions of the hippocampus 24 hours after an immune challenge, which may be attenuated with minocycline. Minocycline did not affect weight gain during development. However, the adult neonatally overfed rats treated with minocycline gained more weight than their saline-treated counterparts without an additional gain in lean mass. Therefore neonatal overfeeding can result in lasting changes to the pro-inflammatory profile in the hippocampus, as well as the ability to respond to an immune challenge and there is potential for neonatal anti-inflammatories to ameliorate these effects.

Poster 93 – Tuesday 6th December

ARIPIPRAZOLE AND HALOPERIDOL ENHANCE DVL3-B-CATENIN SIGNALLING AND DOWNREGULATE NMDA RECEPTORS IN THE VTA AND SN OF RATS

Prof Chao Deng[1], Mr Bo Pan[2]

[1]Antipsychotic Research Laboratory, Illawarra Health and Medical Research Institute, [2]School of Medicine, University of Wollongong

Background: Impaired Wnt-Dvl3-β-catenin signalling and deficits of NMDA receptors are involved in the pathophysiology of schizophrenia. Current antipsychotics mainly bind with dopamine D_2-like and serotonin 5-HT_2 receptors to exert their therapeutic effects, but not directly bind with Wnt and NMDA receptors. The present study investigated the effects of aripiprazole and haloperidol on modulating Dvl-β-catenin signalling and NMDA receptors in the rat brain. **Method:** Rats were orally treated with aripiprazole (0.75 mg/kg), haloperidol (0.1 mg/kg) or vehicle three times per day for 1 week (short-term) or 10 weeks (chronic). The levels of Dvl3, β-catenin and NMDA receptor NR1 and NR2A subunits were measured in the ventral tegmental area (VTA) and substantia nigra (SN) by Western Blots. **Results:** Both 1-week and 10-week treatment of aripiprazole and haloperidol increased the expression of Dvl3 and β-catenin in the SN. Furthermore, 1-week treatment of aripiprazole and haloperidol reduced the protein levels of NMDA NR1 in the VTA. Chronic treatment of aripiprazole decreased the expression of NMDA NR1 and NR2A in the VTA and SN. Chronic treatment of haloperidol also reduced NMDA NR1 expression in the VTA and SN. **Conclusion:** Activation of Dvl3-β-catenin signalling pathway in the SN contribute to the effects of antipsychotics. Additionally, downregulation of NMDA receptors in the VTA and SN may be also involved in the therapeutic efficacy of antipsychotics.

Poster 94 – Monday 5th December

HUMAN ADULT NEUROGENESIS ACROSS THE AGES: AN IMMUNOHISTOCHEMICAL STUDY

Mr Claude Dennis[1], Ms Lisa Suh[1,2], Dr Michael Rodriguez[1], Professor Jillian Kril[1], Dr Greg Sutherland[1]

[1]Discipline of Pathology, Sydney Medical School, University of Sydney, [2]Dementia Research Unit, School of Medical Sciences, University of New South Wales

In lower mammals, neurogenesis continues in two regions of the adult brain, the subventricular zone (SVZ) in the walls of the lateral ventricles where newborn neurons migrate via the rostral migratory stream (RMS) to the olfactory bulb and the subgranular zone of the hippocampus (SGZ) where newborn neurons integrate into the adjacent granule cell layer. The persistence of adult neurogenesis in humans however remains controversial, complicated by limited availability of viable human tissue, and varying methodologies used across different studies. This study used immunohistochemistry and immunofluorescence to characterise markers of cell proliferation and neurogenesis in the SVZ, RMS and SGZ of 23 individuals aged 0.2-59 years using an unbiased, stereological approach. There was a marked decline in the number of proliferating and neurogenic markers, reaching levels no different to that seen in the adjacent parenchyma in both the SVZ and SGZ by four and one years respectively. Furthermore, the phenotype of the proliferating cells within the neurogenic niches changed with age. In infants, proliferating cells co-expressed neural progenitor (epidermal growth factor receptor), immature neuronal (doublecortin and beta III tubulin) and oligodendrocytic (Olig2) markers. However, after three years of age, microglia were the only proliferating cells found in either niche or in the adjacent parenchyma. This marked decline of proliferating cells in the neurogenic niches as well as a shift in the phenotype from neuronal to microglial suggests that alterations in neurogenesis are unlikely to contribute towards the pathogenesis of age-related brain diseases.

Poster 95 – Tuesday 6th December

POSSIBLE ROLE OF PHOSPHODIESTERASES AND CYCLIC NUCLEOTIDE SIGNALING

Dr. Rahul Deshmukh[1], Mr. Ashwani Kumar[1]

[1]Isf College Of Pharmacy, Moga

In recent years, extract of Acorus calamus has been reported to inhibit phosphodiesterases (PDE). The PDE inhibitory potential of β-asarone was evaluated in vitro. The purpose of the current study was to investigate therapeutic potential and neuroprotective mechanisms of β-asarone against intracerebroventricular (i.c.v.) streptozotocin (STZ) induced experimental dementia in rats. STZ was infused bilaterally (3 mg/kg i.c.v.) on alternate days (day 1 and day 3) in rats. Cognitive functions in rats were assessed by Morris water maze and object recognition task. The rats were treated with β-asarone (12.5, 25 and 50 mg/kg) from day 10 to 21 following 1st STZ infusion. On day 22 rats were sacrificed and brains were removed. The cortical and hippocampal brain regions were isolated, homogenized and used for biochemical, neurochemical and neuro-inflammatory investigations. β-asarone showed significant PDE inhibitory potential in vitro. In vivo STZ produced cognitive impairment and increased oxidative stress and inflammatory cytokines whereas; cholinergic hypofunction and a

decrease in monoamines and cAMP and cGMP levels were observed in cortical and hippocampal brain regions. In addition, imbalance in GABA and glutamate levels was also observed in both cortex and hippocampus of STZ-infused rats. β-asarone improved cyclic nucleotide levels and dose dependently attenuated STZ induced cognitive deficit, oxidative stress and neuroinflammation. Neurochemically, β-asarone restored the balance between GABA and glutamate and other neurotransmitters. The observed cognitive improvement following β-asarone in STZ-infused rats may be due to its antioxidant potential and ability to restore brain neurochemistry. Further the current study also indicate important role of PDE inhibition and improved cyclic nucleotide signaling in the beneficial effects of β-asarone.

Poster 96 – Monday 5th December

DELAYED TREATMENT WITH DENTAL PULP STEM CELLS IMPROVES FUNCTIONAL RECOVERY FOLLOWING PHOTOTHROMBOTIC STROKE IN RATS

Mr Wai Ping Yew[1], **Ms Natalia Danica Djukic[1]**, Mr Jaya Jayaseelan[1], Mr FC Choy[3], Dr Xenia Kaidonis[3], Dr Karlea Kremer[3], Professor Richard J Woodman[2], Professor Simon Koblar[3], Professor Neil Sims[1]

[1]Discipline of Medical Biochemistry and Centre for Neuroscience, Flinders University, [2]Flinders Centre for Epidemiology and Biostatistics, School of Medicine, Flinders University, [3]Stroke Research Programme, School of Medicine, University of Adelaide, SAHMRI

Stem cells from multiple sources have been shown to promote recovery in animal models of stroke and are now being used in clinical trials. A population of multipotent stem cells located in the pulp of teeth from adults provides a potentially useful resource for autologous cell treatment following stroke. Previous investigations have shown that dental pulp stem cells (DPSCs) improve recovery in rodent stroke models involving middle cerebral artery occlusion. The aim of the present study was to test whether delayed treatment with DPSCs influences recovery in a model of stroke induced by photothrombosis that targeted the forelimb motor cortex in rats. Three days after stroke induction, DPSCs were injected into the infarct and at a second site immediately caudal to the infarct. Both DPSC-treated and vehicle-treated control rats showed partial recovery of function in the affected forelimb over the subsequent four weeks. However, recovery was significantly better in the DPSC-treated group based on total success and first-trial success in a skilled reaching task as well as a test of forelimb placing in response to vibrissae stimulation ($p<0.05$ for the interaction between treatment and time). The findings demonstrate that DPSCs promote restoration of function in a second model of stroke. This model results in small infarcts that better mimic the relative size of infarcts associated with improved prospects for functional recovery in humans. Thus, the findings add support for the use of DPSCs as a treatment in the post-acute phase of stroke. Supported by the Brain Foundation.

Poster 97 – Tuesday 6th December

TOLL-LIKE RECEPTOR 4 (TLR4) KNOCK-OUT PROMOTES LESION DEVELOPMENT IN A MOUSE MODEL OF ENDOMETRIOSIS

Miss Kelsi Dodds[1], Dr Elizabeth Beckett[1], Miss Jiajun Liu[1,2], Dr Susan Evans[1,3], Professor Mark Hutchinson[1,2]

[1]Physiology, School of Medicine, University Of Adelaide, [2]ARC Centre for Nanoscale BioPhotonics, University of Adelaide, [3]Pelvic Pain SA

Background: Activation of Toll-like receptor 4 (TLR4), expressed by glia within the CNS and peripheral immune cells, has been implicated in the development of persistent pain and inflammation. Our previous data showed that spinal glial expression is altered in a mouse model of endometriosis; a female-specific chronic inflammatory condition that often manifests with pelvic pain. Therefore, we aimed to determine the extent to which TLR4 may mediate this spinal glial alteration and/or the development of endometriosis.
Methods: Endometriosis was induced in 8-14 week-old Balb/C (WT) or TLR4 knock-out (*TLR4*$^{-/-}$) mice by intraperitoneal injection of syngeneic donor endometrium (40 mg) from the proestrus phase. After three weeks development, lesions were stained with H&E, and the number per animal, type, size and location were recorded. The spinal cord was also removed and dissected into segments spanning T13-S1. Spinal sections were then processed via fluorescent immunohistochemistry for glial fibrillary acidic protein (GFAP; astrocytes) and CD11b (microglia), and images analysed using Image J software.
Results: A significantly greater number of endometriosis-like lesions formed in *TLR4*$^{-/-}$ animals (5.2 ± 1.6; $n = 9$) compared to WT (2.2 ± 0.7; $n = 12$) (mean ± SD; $P < 0.001$). The size, location and proportion of lesion types also differed between *TLR4*$^{-/-}$ and WT animals. Spinal immunostaining results in *TLR4*$^{-/-}$ versus WT mice will additionally be discussed.
Discussion: Our early data suggests that activation of TLR4 is important in restricting the development of endometriosis, which may have significant implications for both its prevention and/or future treatment strategies.

Poster 98 – Monday 5th December

A REPORTER TRANSGENE ENGINEERED TO PROVIDE SINGLE CELL RESOLUTION OF THE NONSENSE MEDIATED mRNA DECAY PATHWAY DURING BRAIN DEVELOPMENT

Deepti Domingo[1], Professor Jozef Gecz[1,2,3,4], Dr Lachlan Jolly[2,3]

[1]School of Biological Sciences, The Univeristy Of Adelaide, [2]Robinson Research Institute, The University of Adelaide, [3]School of Medicine, The University of Adelaide, [4]South Australian Health and Medical Research Institute

The nonsense mediated decay pathway (NMD) plays an imperative role in normal brain development. Several key pathway members are implicated in many neuro developmental disorders including childhood intellectual disability. Recently NMD activity has been discovered

to be variable across cell types and display inter-individual variability. Current methods to quantify NMD activity are end point assays which report on total populations of cells, and thus fail to capture dynamic changes resulting from cellular heterogeneity. To overcome these limitations we have engineered a novel fluorescence reporter transgene which can resolve NMD activity at the single cell level. Our single transgene is comprised of three expression cassettes, namely the selection, control and NMD responsive cassettes. The control and NMD responsive cassette co-express distinguishable fluorescent proteins allowing for visual and quantitative real-time output of NMD activity, which are also conducive to standard protein and RNA methods. Using these methods we have shown our NMD reporter transgene to be responsive to NMD inhibition *in vitro*. The selection cassette utilises the Flp/Frt recombination mediated cassette exchange system allowing the transgene to be stably incorporated into the *Col1A1* locus of germ-line competent mouse ES cells. Therefore for the first time this tool allows the creation of NMD reporter mouse lines. This technology can provide visual and quantitative tracking of endogenous NMD activity at a single cell resolution during embryonic brain development and into postnatal life. Furthermore the definition of regions/cell types in the brain most affected by pathogenic NMD disrupting mutations can direct more targeted therapies.

Poster 99 – Tuesday 6th December

ESTRADIOL PROTECTS CORTICAL NEURONS OF MALE C57BL6J MICE AGAINST CHRONIC GLOBAL CEREBRAL HYPOPERFUSION

Madison Zitting[1], Dr. Qing-Hai Lui[1], Dr. Amy Christensen[2], Dr. Robert H. Chow[1], Dr. William Mack[2], **Dr. Rey Dominguez[1,3]**
[1]Keck School of Medicine of USC, [2]Andrus School of Gerontology of USC, [3]University of Tasmania School of Medicine

Damage to cerebral neurons caused by vascular disease is one of the leading causes of disability and death of adults. In addition cerebral vascular disease and white matter lesions are some of the earliest clinical manifestations in sporadic and familial forms of Alzheimer's disease. Estradiol is a sex steroid hormone known to protect the brain against damage associated with cerebral hypoperfusion and neurodegenerative diseases like Alzheimer's. The mechanisms regulating these neuroprotective actions in the brain are not clear and require further investigation. In the present study we introduce a mouse model of cerebral hypoperfusion, the bilateral common carotid artery stenosis model (BCAS), to study in more detail the biological events that cause white matter lesions and to tease apart the mechanism of estradiol action in the brain. The results of our study show that male C57BL6J mice undergoing cerebral hypoperfusion *via* BCAS and then receiving long term oral estradiol treatment perform better on a novelty object recognition behavior task than male BCAS mice receiving placebo treatment. Postmortem Klüver-Barerra staining of brain slices of male mice shows that estradiol treatment protected the white matter tracts of the corpus callosum and cingulate bundle in the estradiol treated group. Immunofluorescent analysis of slices showed that estrogen receptor-α (ERα) and the extracellular signal regulated kinase (ERK) pathway were also active in the estradiol treated group. Our study demonstrates that the BCAS model is amendable to study estradiol-mediated protection against cerebral hypoperfusion and damage to white matter tracts of the brain.

Poster 100 – Monday 5th December

A ZEBRAFISH MODEL OF THE MND LINKED C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION.

Dr Emily Don[1], Dr Elinor Hortle[1], Ms Serene Gwee[1], Ms Jessica Sultana[1], Dr Sharron Chow[1], Professor Roger Chung[1], A/Prof Karl Clark[2], Professor Garth Nicholson[1], A/Prof Julie Aktin[1], Professor Stephen Ekker[2], Dr Nicholas Cole[1]

[1]Macquarie University, [2]Mayo Clinic Cancer Center

Motor Neuron Disease (MND) is a fatal neurodegenerative disease for which there are no effective treatments. Recently, the most common genetic cause of MND was identified as a hexanucleotide repeat expansion in a non-coding region of the gene C9orf72. While healthy individuals carry 2-23 repeats, MND patients can have up to 700-1600 repeats. In patients, the large repeat expansions cause a down regulation of C9orf72 expression, abnormal RNA foci and dipeptide repeat proteins (DPRs). However, it is currently unknown how the hexanucleotide repeat expansion leads to the loss of motor neurons and the development of MND. Therefore, there is a great need for models in which to study the C9orf72 hexanucleotide repeat expansion. Whilst approaches using cell cultures and mouse models of the repeat expansion have yielded promising results, the optical transparency and ease of transgenesis make zebrafish an ideal model to observe, in real time, the downstream effects of the C9orf72 repeat expansion.
We have previously inserted normal and expanded copies of the C9orf72 repeat expansion into zebrafish. Expanded copies of the hexanucleotide repeat result in significantly more aberrantly branched motor neurons and the early death of the transgenic zebrafish compared to non-transgenic sibling controls. We are now examining if the C9orf72 repeat expansion DPRs could be responsible for the observed motor neuron defects. In combination with live-microscopy, we are exploring mosaic expression of the individual DPRs to model the C9orf72 hexanucleotide expansion in zebrafish to allow for observation of disease progression at a molecular and cellular morphology level.

Poster 101 – Tuesday 6th December

IDENTIFICATION OF NOVEL MOLECULAR ELEMENTS MEDIATING NEURONAL RESPONSE TO REACTIVE OXYGEN SPECIES.

Miss Alessandra Donato[1], Dr Sean Coakley[1], Miss Eva Kaulich[1], Dr S. A. Kim[2], H. Lee[2], Prof. Hang Lu[2], Assoc/Prof. Massimo A. Hilliard[1]

[1]Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, [2]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology

Reactive oxygen species (ROS) include a number of molecules involved in many biochemical activities of cells, such as cell signaling and homeostasis. However, accumulation of ROS leads to oxidative stress that is recognized as a pathological factor in several neurodegenerative conditions. To date, the molecular mechanisms of neurodegeneration upon ROS accumulation are poorly understood. KillerRed is a genetically encoded red fluorescent molecule that is able to generate ROS upon illumination with green light, in a temporally and spatially controlled manner. Here we use the nematode *C. elegans* expressing KillerRed selectively in the six mechanosensory neurons, as an experimental model system to investigate the cellular response to ROS damage *in vivo*. Using a candidate gene approach, we first investigated the role of molecule involved in cell death mechanisms and found that lack of necrotic genes does not suppress the ROS-induced cell death, suggesting that other pathways are involved in this process. Moreover, we uncovered redundant and compensatory effects of the ROS-scavenging enzymes superoxide dismutases (SODs) and catalases. Finally, we developed a new multi-illumination platform that allows forward genetic screening to be performed for the first time. We identified three novel mutants presenting significantly increased neuronal damage after KillerRed activation. The results obtained from our studies have the potential to reveal the neuronal-specific components that regulate the response to ROS-induced damage and untangle some of the pathological mechanisms that threaten our nervous system.

Poster 102 – Monday 5th December

BDNF MAINTAINS NORMAL ESTROUS CYCLING – RELEVANCE TO SCHIZOPHRENIA

Dr Xin Du[1], Ms Cushla McCarthny[2], Ms Anna Schroeder[2], Mr Michael Notaras[2], Ms Adrienne Grech[1], Dr Rachel Hill[1]

[1]Monash University, [2]The Florey Institute of Neuroscience and Mental Health

Background:
Recent clinical evidence suggests that a large proportion of women with schizophrenia exhibit abnormal menstrual cycles and reduced circulating estradiol levels, which may contribute to symptoms such as cognitive decline. While this phenomenon is partially accounted for by antipsychotic-induced hyperprolactinaemia, it is likely induced by innate pathophysiological changes related to the disease. We propose that a reduction of brain-derived neurotrophic factor (BDNF), as observed in schizophrenia, may be sufficient in inducing pathological changes in the hypothalamic-pituitary-gonadal (HPG) axis, thereby leading to alterations of menstrual cycling and reductions in estrogen production.
Methods:
We used both wild-type and BDNF heterozygous female mice, which express ~50% of normal BDNF levels. To assess estrous cycling, vaginal smears were performed 2-3 times a day for 3 weeks from 7-9 weeks of age. Furthermore, brain tissue was collected in separate cohort of female mice at both low and high estradiol-expressing phases of the estrous cycle to assess expression levels of key regulators of HPG-axis function, including GnRH receptor protein expression in both the hypothalamus and the hippocampus.
Results:
Female BDNF heterozygous mice exhibit dysregulation of their estrous cycle in a remarkably similar manner to that reported in female schizophrenia patients. Where the schizophrenia patients showed elongation of menstrual cycle, the BDNF heterozygous mice show increased cycle lengths compared to wild-type mice. Particularly, in the BDNF heterozygous mice there is elongation of the pro-estrus.

Poster 103 – Tuesday 6th December

PERIPHERAL AND CENTRAL NEUROINFLAMMATORY CHANGES AND PAIN BEHAVIOURS IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

Mr Samuel Duffy[1], Dr Chamini Perera[1], Mr Preet Makker[1], Dr Justin Lees[1], A/Prof Pascal Carrive[1], Dr Gila Moalem-Taylor[1]
[1]University Of New South Wales

Pain is a widespread and debilitating symptom of multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system. Although central neuroinflammation and demyelination have been implicated in MS-related pain, the contribution of peripheral and central mechanisms during different phases of the disease remains unclear. In this study, we used the animal model experimental autoimmune encephalomyelitis (EAE) to examine both stimulus-evoked and spontaneous pain behaviours over the course of chronic disease, and assessed potential underlying pathological mechanisms. Neuroinflammation was assessed using a combination of flow cytometry and immunohistochemistry. We found that mechanical allodynia of the hind paw preceded the onset of clinical EAE, but was unmeasurable at clinical peak. This mechanical hypersensitivity coincided with an increased microglial activation confined to the dorsal horn of the spinal cord. The development of facial mechanical allodynia also emerged in pre-clinical EAE, persisted at the clinical peak, and corresponded with pathology of the peripheral trigeminal afferent pathway. This included T cell infiltration and specific damage to myelinated neurons, both of which arose prior to overt central lesion formation. Measurement of spontaneous pain using the mouse grimace scale showed significantly increased facial grimacing in mice with EAE during clinical disease. This was associated with multiple peripheral and central neuroinflammatory changes including a decrease in myelinating oligodendrocytes, increased T cell infiltration and macrophage/microglia and astrocyte activation. Overall, these findings suggest that different pathological mechanisms underlie stimulus-evoked and spontaneous pain in EAE, and that these behaviours predominate in unique stages of the disease

Poster 104 – Monday 5th December

MULTICOLOUR ANALYSIS OF OLIGODENDROCYTE MORPHOLOGY AND INTERACTIONS WITH BRAINBOW

Laura Dumas[1], Céline Heitz-Marchaland[2], Stéphane Fouquet[2], Ueli Suter[3], Jean Livet[2], Caroline Moreau-Fauvarque[2], Tobias David Merson[1], Alain Chédotal[2]

[1]Australian Regenerative Medicine Institute, Monash University, [2]Institut de la Vision, [3]Institute of Molecular Health Sciences

Oligodendrocytes are the myelinating cells of the central nervous system. Although the molecular mechanisms that regulate oligodendrocyte production are increasingly well understood, little is known about the cellular dynamics responsible for coordinating how myelin internodes are organised topographically within white matter. This is mainly due to the lack of suitable methods for identifying individual myelin internodes produced by adjacent oligodendrocytes. To overcome this technical hurdle, we adopted a highly innovative and powerful imaging technique in order to simultaneously visualise many individual oligodendrocytes with distinct combinations of fluorescent reporter proteins. This method is based on Brainbow, a transgenic system for stochastic expression of multiple genes encoding spectral variants of GFP following random Cre-*lox* recombination events. To obtain multicolour labelling of individual oligodendrocytes, we crossed *CAGbow* mice with *PLP:Cre^{ERT2}* transgenic mice. The density and colour/hue of recombined oligodendrocytes was tamoxifen-dependent. High doses of tamoxifen produced dense multicolour labelling of the myelinating network and revealed the contact between neighbouring oligodendrocytes before and after nodes of Ranvier. Interestingly, in instances where adjacent oligodendrocytes expressed different coloured reporter proteins that were both membrane-targetted, it was possible to demonstrate that neighbouring oligodendrocytes myelinate a common set of axons. Our data provide the first step towards a more comprehensive understanding of the white matter network at a cellular resolution that could provide new insights into demyelinating diseases. We are currently refining our labelling approach to enable the expression of only membrane-targeted Brainbow variants in order to map the complete topographic distribution of myelin internodes in white matter.

Poster 105 – Tuesday 6th December

ADULT SKIN-DERIVED NEURAL PRECURSORS – A CANDIDATE FOR AUTOLOGOUS NEUROREPLACEMENT THERAPIES

Dr Thomas Duncan[1], Dr Aileen Lowe[1], Dr Joyce Siette[1], A/Prof Kuldip Sidhu[2], Prof Fred Westbrook[3], Prof Perminder Sachdev[4], Dr Billy Chieng[5], Dr Trevor Lewis[6], A/Prof Ruby Lin[7], Dr Vladimir Sytnyk[8], A/Prof Michael Valenzuela[1]

[1]Regenerative Neuroscience Group, Brain and Mind Centre, University of Sydney, [2]Stem Cell Laboratory, University of New South Wales, [3]School of Psychology, University of New South Wales, [4]Centre for Healthy Brain Ageing, University of New South Wales, [5]Behavioral Neuroscience Laboratory, Brain and Mind Centre, University of Sydney, [6]School of Medical Sciences, University of New South Wales, [7]Asbestos Diseases Research Institute, Bernie Banton Centre, Concord Hospital, [8]School of Biotechnology and Biomolecular Science, University of New South Wales

The isolation and culture of neural stem cells shows promise for autologous stem cell therapies aimed at the regeneration of depleted or dysfunctional neuronal circuits in patients with neurodegenerative disorders. The rapid clinical translation of this research has however been limited by the frequently reported propensity for glial differentiation *in vitro* and *in vivo*, or due to a technical reliance on genetic modifications.

We utilize a two-step method combining initial neurosphere selection with adherent monolayer expansion to generate high yields of *P75-Nestin-CD133*-positive neural precursor cells from adult canine skin without the use of genetic manipulation. Following differentiation *in vitro*, these cells express high levels of neuron specific markers such as *βIII-tubulin (96%) and MAP2 (74%),* with no glial marker expression. After transplantation into the hippocampi of aged rats, these cells survive, migrate extensively, and mature structurally and functionally into electrophysiologically active neurons capable of establishing synaptic connectivity with host hippocampal circuitry. Furthermore, following transplantation, significant age-related spatial memory impairments were reversed, with performance restored back to levels equivalent with young animals.

Skin derived neural precursors are rate-limited, striking a balance between unlimited self-renewal and terminal differentiation. They are easily accessible, highly homogenous in culture, and highly restricted to a CNS neuronal fate *in vivo*. Our success in reversing age-related memory impairment in rats has paved the way for a current therapeutic study in dogs, utilizing autologous neural precursors in the treatment of canine cognitive dysfunction, a neurodegenerative disorder analogous to human Alzheimer's dementia.

Poster 106 – Monday 5th December

EFFECT OF MATERNAL IMMUNE ACTIVATION AND SEX ON ELECTROPHYSIOLOGICAL FEATURES RELATED TO SCHIZOPHRENIA

Miss Ariel Dunn[1,3], Dr Lauren Harms[1,3], A/Prof. Juanita Todd[1,3], Dr Ross Fulham[1,3], Dr Aaron Wong[1,3], Prof. Deborah Hodgson[1,3], Prof. Ulrich Schall[2,3], Emeritus Prof. Patricia Michie[1,3]

[1]School of Psychology, University of Newcastle, [2]School of Medicine and Public Health, University of Newcastle, [3]Priority Research Centre for Brain and Mental Health Research, University of Newcastle

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.

Poster 107 – Tuesday 6th December

MODELLING THE EFFECT OF AMYLOID-BETA IN OLIGODENDROCYTE DIFFERENTIATION AND MATURATION

Samuel T Dwyer[1], Dr. Jacqueline YK Leung[1], Dr. Matthew Kirkcaldie[1], Dr. Anna E King[1], Prof. James C Vickers[1]

[1]Wicking Dementia Research And Education Centre, University of Tasmania

Oligodendrocytes (OL) are a supporting cell in the brain, whose main function is to produce myelin. Myelin acts as insulation to the axons, allowing rapid transmission of action potential as well as maintaining their health and structure. The loss of myelin has been reported in Alzheimer's disease (AD), one of the most commonly occurring forms of dementia. The reported myelin loss has often been considered as a secondary event, which occurs as a result of the loss of axons, however, recent studies have shown evidence that myelin loss could be an independent event in AD, resulting from the toxicity of beta-amyloid. To determine the effect of beta-amyloid on oligodendrocytes, we first established and characterized a primary culture model to grow oligodendrocytes to specific stages of differentiation (OPC, Immature-OL, Mature-OL) using stage-specific media and following a recently published protocol. Immunocytochemical analysis was used to characterize the different developmental stages present in the cultures. Our results demonstrated that application of Mature-OL media to induce oligodendrocyte maturation resulted in significantly ($p<0.05$) more oligodendrocytes immunopositive for the myelin marker proteolipid protein (PLP) than OPC or Immature-OL media following 5 days in culture, with >70% cells immunoreactive. This demonstrates that our new is able to generate high percentages of oligodendrocytes at specific stages of development. This model will now be utilized to characterized the effect of extracellular Aβ on oligodendrocyte development *in vitro*.

Poster 108 – Monday 5th December

CHROMATIC OPPONENCY AND RECEPTIVE FIELD PROPERTIES OF BLUE-ON (S-CONE) CELLS IN PRIMATE LATERAL GENICULATE NUCLEUS

Dr. Calvin Eiber[1,2,3], Dr. Alexander Pietersen[1,2,3], Natalie Zeater[1,2,3], Dr. Solomon Samuel[4], Prof. Paul Martin[1,2,3]

[1]Bilson Laboratory, Save Sight Institute, University Of Sydney, [2]ARC Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]School of Medical Sciences, The University of Sydney, [4]Department of Experimental Psychology, University College London

Purpose: In retina of diurnal primates, a small bistratified ganglion cell class shows "blue-on/yellow-off" receptive field properties. This arises from on-type input from short-wave sensitive (S) cones and off-type input from medium/long-wave sensitive (ML) cones (Dacey and Lee, Nature, 1994). These cells project to the koniocellular layers of the LGN, where blue-on/yellow-off receptive fields are commonly encountered. It is generally assumed that responses to achromatic stimuli in these cells are linearly proportional to the summed activity of the S-cones within the receptive field, less summed activity of the ML cones within a corresponding field. We tested this hypothesis. **Methods:** Extracellular recordings of isolated unit responses (N = 45) to cone-selective and achromatic drifting gratings were made in Sufentanil-anesthetised marmosets (N = 19). Gaussian receptive fields were fit to spatial frequency tuning curves, and a predicted achromatic spatial tuning curve was generated from the S- and ML-cone-isolating gratings. Akaike information criterion was applied to identify the best model. **Result and conclusion:** The best model consisted of a linearly summing single Gaussian field in each of S and ML, as compared to models with centre-surround organization in S or ML, or models where S and ML shared a single receptive field ($p < 0.05$ in both cases, Tukey-Kramer multiple comparisons test). The prediction error for the achromatic data was less than 5 impulses/s in 70% of the cells. We conclude that a linear sum of opponent cone inputs explains achromatic responses of blue-on cells when S and ML have different space constants.

Poster 109 – Tuesday 6th December

REGIONAL CHANGES IN MOUSE MOTOR CORTEX EXCITABILITY AFTER FOCAL STROKE.

Assoc Prof Ruth Empson[1], Dr Emmet Power[1], Miss Emma Gowing[2], Dr Andrew Clarkson[2,3]

[1]Department of Physiology University Of Otago, [2]Department of Anatomy University of Otago, [3]Faculty of Pharmacy, The University of Sydney

About 9000 people in New Zealand have a stroke every year (Stroke.org.nz). It is the third leading cause of mortality and, for those who survive, it can cause a range of permanent disabilities. In this project we aim to understand changes in functional connectivity in the motor cortex after stroke in order to identify mechanisms that can influence recovery.

Here, we use a transgenic mouse expressing a Voltage Sensitive Fluorescent Protein (VSFP, also called Butterfly) in cortical layer 2/3 neurons. We induced a focal stroke (or sham) in the M1 (motor) region of the prefrontal cortex, approximately 2mm in diameter, and recorded Local Field Potentials (LFP) and fluorescence based voltage imaging signals from layer 2/3 two weeks later.
We observed reduced excitability in response to increasing Layer 5a stimulation in layer 2/3 of both M1, approximately 350µm ventral to the stroke border ($P<0.05$, 2-way ANOVA) and M2 (pre-motor), approximately 1mm ventral to the stroke border ($P<0.005$, 2-way ANOVA) compared to sham controls.
The decreased excitability of the M2 region highlights synaptic reorganisation in a distal cortical region not directly affected by the initial injury. Whether this hypo-excitability during the second week post-stroke helps or hinders recovery remains to be determined.

Poster 110 – Monday 5th December

Nav1.8 IS REQUIRED FOR MECHANOSENSATION IN HYPERSENSITIVE COLONIC AFFERENTS AND Nav-INDUCED COLONIC PAIN IN VIVO

Ms Andelain Erickson[1], Dr Joel Castro[1], Dr Sonia Garcia-Caraballo[1], Dr Luke Grundy[1], Dr Andrea M Harrington[1], Ms Jessica Maddern[1], A/Prof Grigori Y Rychkov[1], Prof Richard J Lewis[2], Dr Irina Vetter[2], A/Prof Stuart M Brierley[1,3]

[1]Visceral Pain Group, Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, The University Of Adelaide, SAHMRI, [2]Institute for Molecular Bioscience, The University of Queensland, [3]Visceral Pain Group, Flinders University

Objective: Voltage-gated sodium (Na_v) channels regulate action potential generation and cell membrane excitability in sensory neurons, and are implicated in several pain phenotypes. In this study, we examined the contribution of $Na_v1.8$ in colonic pain signaling in a mouse model of chronic visceral hypersensitivity (CVH). **Methods:** Gene expression profiles of $Na_v1.1$-$Na_v1.9$ were obtained by Quantitative RT-PCR in dorsal root ganglia (DRG) at spinal levels T10-S1 from healthy and CVH mice. *In vitro* electrophysiological recordings were performed on colonic nociceptors from healthy and CVH mice in the presence and absence of the $Na_v1.8$-selective antagonist A-803467. *In vivo* pain behavior studies were performed in mice with an intra-colonically administered pan-Na_v channel activator in the presence or absence of A-803467. **Key findings:** $Na_v1.8$ was consistently the most abundant Na_v isoform across T10-S1 DRG, with significant upregulation in CVH DRG neurons at levels T12 and T13 of the thoracolumbar region innervating the colon ($p<0.001$, $N = 6$). *In vitro* electrophysiology recordings showed that A-803467 administration caused a significant reduction in CVH colonic nociceptor mechanosensitivity ($p<0.05$, $n = 8$), whereas nociceptors from healthy mice were not affected by $Na_v1.8$ inhibition. *In vivo* intra-colonic administration of a pan-Na_v channel activator significantly increased pain behaviors, which were fully reversed by co-administration with A-803467 ($p<0.05$, $N = 6$-12 animals/group). **Conclusion:** $Na_v1.8$ upregulation in thoracolumbar DRG appears to contribute to CVH colonic nociceptor hypersensitivity. As shown by *in vivo* studies, targeting $Na_v1.8$ could be beneficial in the treatment of chronic colonic pain syndromes.

Poster 111 – Tuesday 6th December

EFFECTS OF COCHLEAR IMPLANT USE ON BINAURAL PROCESSING

A/Prof James Fallon[1,2], Dr Andrew Wise[1,2], Prof Dexter Irvine[1,2]

[1]Bionics Institute, [2]Department of Medical Bionics, University of Melbourne

The effects of chronic bilateral cochlear implant use on binaural processing are unclear. We therefore examined the effects of chronic bilateral cochlear implant use interaural time difference (ITD) and interaural level difference (ILD) sensitivity in long-term neonatally deafened animals.
Three groups of cats were used: two normal hearing controls (NHC), two neonatally profoundly deafened unstimulated cats (NDUS) and four neonatally profoundly deafened cats that received approximately 6 months of bilateral intra-cochlear electrical stimulation from clinical cochlear implants and speech processors (NDS). Single-unit responses ($n = 110$, 60, 86 for the NHC, NDUS, and NDS groups, respectively) to electric binaural stimulation with a range of ITDs and ILDs were recorded from the central nuclei of the inferior colliculus bilaterally, using 32-channel silicon arrays (NeuroNexus).
ITD sensitivity was significantly poorer in both the neonatally deafened groups compared to the normal hearing animals (Kruskal-Wallis test, $p < 0.05$), and there was no difference between the stimulated and unstimulated groups ($p > 0.05$). ILD sensitivity was not different between the groups ($p > 0.05$).
The use of bilateral clinical cochlear implants does not prevent/reverse the degradation in ITD processing that occurs following long-term deafness from a young age. Whether experience with appropriate ITD cues would improve ITD processing still needs to be examined. ILD processing is largely unaffected by either long-term deafness or chronic stimulation.
Funded by the NH&MRC and the GPRWMF. The Bionics Institute acknowledges the support it receives from the Victorian Government through its Operational Infrastructure Support Program.

Poster 112 – Monday 5th December

OPTICAL TRAPPING OF OTOLITHS IN ZEBRAFISH PRODUCES FICTIVE VESTIBULAR STIMULI AND COMPENSATORY BEHAVIOURS

Itia Favre-bulle[1], DR Alex Stilgoe[1], Gilles Vanwalleghem[1], Lucy Heap[1], Dr Andrew Thompson[1], Pr Halina Rubinztein-Dunlop[1], A/Prof Ethan Scott[1]

[1]University Of Queensland

The mechanisms by which the brain perceives and processes vestibular information are poorly understood. This is because popular approaches for observing brain activity (MRI, electrophysiology, and microscopy) are poorly suited to moving subjects. We have developed an experimental preparation in which we use an infrared laser to perform optical trapping on the otoliths (earstones) of intact, behaving, immobilised zebrafish larvae. Using this preparation, we can apply forces to the otoliths, simulating physical acceleration of the animal, although it remains stationary. By tracking tail and eye movements of head-embedded larvae in this preparation, we have begun to explore the relationship between forces placed on the otoliths and behavioural responses to the perceived vestibular stimuli. We find that trap strength correlates with the magnitude of postural changes in the tail, and that stronger traps also trigger forward swimming. Trapping also induces movements in the eyes, apparently to compensate for a perceived rolling motion. We will now perform functional imaging using genetically encoded calcium indicators and a house-build selective planar illumination microscope to map out the circuit dynamics that lie between our vestibular stimulation and the behaviours that it elicits.

Poster 113 – Tuesday 6th December

CHRONIC LIPID PEROXIDATION FOLLOWING REPEATED MILD TRAUMATIC BRAIN INJURY

Ms Brooke Fehily[1], Mr Nathanael Yates[1], Mrs Carole Bartlett[1], Mr Stephen Lydiard[1], Assoc Prof Melinda Fitzgerald[1]

[1]Experimental and Regenerative Neurosciences, School of Animal Biology, The University of Western Australia

Following mild traumatic brain injury (mTBI), some patients go on to experience long-term cognitive impairments and additional mild impacts can result in exacerbated and persisting negative outcomes. Characterising chronic pathology related to inflammation and oxidative stress following repeated mTBI may facilitate the development of therapeutic strategies to limit long term functional deficits. Using a closed-head weight-drop model of repeated mTBI, a total of 0, 1x, 2x or 3x mTBIs were delivered to adult female rats at 24 hour intervals under isofluorane anaesthesia; no skull fractures were observed. Sham injuries were delivered in place of mTBI, such that all animals received three anaesthesia exposures. Outcomes were assessed at 3 months following the first mTBI. Motor function was assessed using a Ladder walk paradigm and no significant deficits were identified ($p > 0.05$), consistent with current literature. The number of Iba1+ microglia in the corpus callosum did not change as a consequence of repeated mTBI ($p > 0.05$). Oxidative stress was assessed using immunohistochemical indicators of lipid peroxidation. 4-hydroxynonenal immunointensity above an arbitrary threshold was increased in ventral brainstem for all mTBI groups, compared to sham controls ($p \leq 0.05$). Similarly, acrolein immunointensity above threshold in the cerebellum was increased following 1x and 3x mTBI, relative to sham ($p \leq 0.05$). Chronic lipid peroxidation in deep brain structures was revealed in the absence of increased microglial numbers in white matter tracts of the corpus callosum. Further studies exploring cognitive function and neuroinflammatory and oxidative changes in hippocampal and sub-cortical structures are ongoing.

Poster 114 – Monday 5th December

WHOLE TRANSCRIPTOME ANALYSIS SHOWS INVOLVEMENT OF IMMUNE RESPONSE AND INFLAMMATION IN TDP-43-RELATED FRONTOTEMPORAL LOBAR DENEGERATION (FTLD-TDP)

Guinevere F. Lourenco[1,2], Boris Guennewig[3], James D. Mills[4], John S. Mattick[3], Antony A. Cooper[3], Woojin S. Kim[1,2], John B. Kwok[1,2], Carol Dobson-Stone[1,2], Matthew C. Kiernan[1,2], John R. Hodges[1,2], Jillian J. Kril[5], Lars M. Ittner[1], Jürgen Götz[6], Rachel H. Tan[1,2], Caroline Janitz[7], Michael Janitz[4], Glenda M. Halliday[1,2]

[1]School of Medical Sciences, UNSW Australia, [2]Neuroscience Research Australia (NeuRA), [3]Garvan Institute of Medical Research, [4]School of Biotechnology and Biomolecular Sciences, UNSW Australia, [5]Sydney Medical School, The University of Sydney, [6]Queensland Brain Institute, The University of Queensland, [7]University of Western Sydney

Frontotemporal lobar degeneration (FTLD) is characterized by the progressive changes in behavior and personality, executive dysfunction, and decline of language skills. Although histopathologically diverse, the majority of FTLD cases have abnormal cytoplasmic accumulation of the nuclear RNA-binding protein TDP-43 (FTLD-TDP). TDP-43 plays crucial roles in several steps of RNA metabolism, but the extent to which TDP-43 pathology impacts the brain transcriptome during disease remains unclear. In this study, we used strand-specific RNA-Seq technology to investigate changes in the transcriptome profile of FTLD-TDP human brain tissue from the NSW Brain Banks (following institutional ethics and tissue approvals). Our results revealed that genes differentially expressed (DE) in FTLD-TDP are mostly upregulated (69%) and protein-coding genes (78%). We found that cell death and survival, cell signaling and interaction, protein synthesis, and molecular transport are the most enriched cellular functions altered in FTLD-TDP, and that immune response and inflammation-related pathways are particularly affected in this disease. We analyzed publically available single-cell RNA-Seq datasets derived from healthy brain cell types, and found a greater level of dysregulation among microglia- and neuron-specific genes (10 and 9 genes dysregulated, respectively). Furthermore, we saw that microglia- and astrocyte-specific genes are mostly upregulated in FTLD-TDP, while genes specifically expressed in neurons and oligodendrocytes show a trend for downregulation. Together, our data suggest that immune response and inflammation might play a major role in FTLD-TDP, and that the impact is greatest on the transcriptomes of neurons and microglia.

Poster 115 – Tuesday 6th December

CARBONIC ANHYDRASE VII EXPRESSION IN HYPOXIC ISCHAEMIC ENCEPHALOPATHY

Dr Matthew Fielder[1], Ms Stephanie Miller[1], Professor Paul Colditz[1], Dr Tracey Bjorkman[1]

[1]Perinatal Reseach Centre, UQ Centre For Clinical Research

Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland, Herston, Qld 4029

Seizures following hypoxic-ischaemic injury have been shown to be an independent cause of neuronal damage in the newborn. These seizures are related to the immature, excitatory circuits in the neonatal brain and to neonatal brain pH. A key determinant of neonatal brain pH is the bicarbonate buffering system, and in particular, the expression of certain isoforms of carbonic anhydrase. Consequently, the level of expression of these catalytic enzymes impact on neonatal brain pH and may play a role in neonatal seizure generation following hypoxic-ischaemic injury. Carbonic anhydrase inhibitors have been used as anticonvulsants in the adult population, however, their non-specific nature reduces their effectiveness in neonatal patients.
A piglet model of hypoxic-ischaemic encephalopathy was used to determine the expression of carbonic anhydrase VII in the neonatal brain. Three brain regions were analysed, the parietal cortex, hippocampus, and thalamus. Carbonic anhydrase VII expression was determined using ELISA in three groups; control piglets, hypoxic-ischaemic piglets without seizures, and hypoxic-ischaemic piglets with seizures. Carbonic anhydrase VII expression was found to be upregulated in the hypoxic group with seizures compared to the control group in all three brain regions, and the upregulation in the thalamus was significant.
Alterations in the expression of carbonic anhydrase VII in hypoxic-ischaemic encephalopathy is likely to impact on neonatal brain pH, and hence on the excitability and susceptibility of the neonatal brain to seizures. Isozyme specific carbonic anhydrase inhibitors may provide a therapeutic advantage in the neonatal population.

Poster 116 – Monday 5th December

EVALUATION OF BINARISATION ALGORITHMS FOR IMAGE ANALYSIS OF GLIAL CELLS IN EX VIVO SLICE CULTURES

Dr. Una Fitzgerald[1], Ms Sinead Healy[1], Dr. Jill McMahon[1]

[1]Galway Neuroscience Centre, School of Natural Science, National University of Ireland

Galway Neuroscience Centre, School of Natural Sciences, National University of Ireland, Galway, Ireland.

Despite recent advances, an agreed standard for digital image analysis of glia has not been described. Stringent evaluation and detailed reporting of such protocols, notably image segmentation and feature extraction, is seldom included in publications reporting data derived from image analysis, raising concerns about the repeatability and definitiveness of reported information. To address this issue, cultured *ex vivo* slices were immunolabelled with cell-specific markers: GFAP for astrocytes; Olig2 for oligodendrocytes; IBA1 for microglia. They were then imaged on a laser scanning confocal microscope and Z-projection fluorescent images acquired and processed using FIJI software version 2.0.0-rc-49/1.51d. The quality, accuracy, specificity and sensitivity of 25 available algorithms for Z-stack image representation, image segmentation and feature extraction were evaluated. In a qualitative assessment, we found that the *maximum* intensity projection was the best for concisely, sensitively and accurately conveying z-stack information in 2D images of all glial cell types. Next, we determined that *automatic* thresholding-based image binarisation and feature extraction out-performed three different single user-chosen thresholds. In what we believe is the first reported application of FIJI's BIoVoxxel plugin to compare quantitatively different algorithms when applied to images of stained glial cells, we confirmed a statistical difference in quality, accuracy, specificity and sensitivity of 16 global and 9 local algorithms ($p<0.05$) when compared to each other. Post-hoc comparison using Dunnett's test indicated that the *Li* and *Huang* algorithms may be considered as first-line segmentation algorithms in the analysis glia stained with standard cell-specific antibodies. The *Otsu* algorithm may produce false positive results ($p<0.0.05$).

Poster 117 – Tuesday 6th December

IMPACT OF ENDOPLASMIC RETICULUM STRESS SIGNALING ON OLIGODENDROCYTE MATURATION

Dr. Una Fitzgerald[1], Ms Michelle Naughton[1], Dr. Jill McMahon[1]

[1]Galway Neuroscience Centre, School of Natural Sciences, National University of Ireland, Galway

Many integral membrane proteins destined for insertion into myelin are synthesised and trafficked through the endoplasmic reticulum (ER). Dramatic expansion of the oligodendrocyte (OL) membrane during myelination likely places an extraordinary burden on the ER, triggering ER stress or the unfolded protein response (UPR). We recently reported a significant upregulation of activated ER stress sensors p-IRE 1 and ATF6 and downstream proteins associated with ER stress signaling in developing white matter tracts (Naughton et al, 2015). To more accurately identify which aspects of oligodendrocyte differentiation might be regulated by the UPR, *in vitro* cultures of oligodendrocyte precursor cells (OPCs) were treated with guanabenz and 4μ8C. Guanabenz inhibits de-phosphorylation of eIF2α, thus, prolonging protein translation arrest, and it caused a significant reduction in the numbers of mature oligodendrocytes at 72 hours, compared to untreated cells ($p<0.01$). OPC exposure to 4μ8C, an inhibitor of IRE1 signaling, significantly impaired differentiation at 24, 48 and 72 hours ($p<0.001$). An early pro-differentiating effect of guanabenz ($p<0.01$) was negated by 4μ8C when applied in combination and combined treatment was only significantly different from control at 72 hours ($p<0.05$). A dose-dependent decrease in MOG, PLP and MBP mRNA levels was observed in cultures treated with 2, 5 and 10μM 4μ8C, but no changes in UPR-associated transcripts (XBP1u, XBP1s, PDI, ATF6, GRP94, GRP78) were detectable. Up to 6-fold increases in myelin gene expression in differentiating O2A oligodendrocyte cell line was not accompanied by significant changes in UPR-associated transcripts. This data points towards an adaptable UPR within maturing oligodendrocytes

Poster 118 – Monday 5th December

TRKB AGONIST PROMOTE MYELIN REPAIR IN THE BRAIN

Dr Jessica Fletcher[1], Ms Rhiannon Wood[1], Ms Alexa Prawduik[1], Ms Jacqueline Nguyen[1], Dr Junhua Xiao[1], Dr Simon Murray[1]

[1]*The University Of Melbourne*

There is pressing clinical need to develop novel therapies for demyelinating diseases like Multiple Sclerosis (MS) that promote myelin repair and complement existing immunomodulatory treatments. We have shown that the neurotrophin, brain-derived neurotrophic factor (BDNF) enhances myelination through activation of TrkB receptors expressed by oligodendrocytes. To test if selective TrkB activation promotes remyelination in the brain, we infused known TrkB agonists, TDP6, LM22A4 and 7,8'-dihydroxyflavone (DHF) into the lateral ventricles of adult mice fed 0.2% cuprizone for 6 weeks to induce demyelination. Following infusion for 7 days, all three TrkB agonists significantly increased the levels of myelin proteins MBP and MAG (p=0.0012 and p=0.005, respectively) compared to vehicle controls. Examining oligodendrocyte populations, all TrkB agonists significantly increased the total number of oligodendroglia (p=0.0013). However, differential effects were observed with TDP6 and LM22A4 both increasing the density of intermediate and mature myelinating oligodendrocytes (p=0.0017 and p=0.011, respectively), while DHF resulted in a significant increase in oligodendrocyte progenitor cells (p=0.021). TrkB phosphorylation and ultrastructural myelin changes are being assessed. Our results are indicative that binding efficiency may alter the differentiation potential of TrkB activation and that overall stimulating TrkB can promote myelin repair following central nervous system demyelination.

Poster 119 – Tuesday 6th December

CNS REWARD PATHWAYS IN ANOREXIA NERVOSA: INSIGHTS FROM A RAT MODEL.

Dr Claire J Foldi[1], Ms Laura K Milton[1], Professor Brian J Oldfield[1]

[1]*Monash University*

Patients suffering anorexia nervosa (AN) become anhedonic; unable or unwilling to derive normal pleasures and avoid rewarding outcomes, most profoundly in food intake. The activity-based anorexia (ABA) model allows investigation into the underlying neurobiology of AN, especially because it displays many characteristics in common with the human condition, including anhedonia. We aim to exploit this model to highlight the importance of CNS reward in the maintenance of body weight. We hypothesise that increasing neuronal activity of circuits with predicted involvement in the anhedonia/reward pathways of ABA will prevent associated weight loss.
Female rats (*n*=24; 6 weeks old) underwent separate bilateral stereotaxic injections of canine adenovirus-2-Cre (CAV-2-Cre) and activating DREADDs (AAV-hSyn-DIO-hM3D(Gq)-mCherry) into the NAcc and VTA, respectively. DREADDs reorient in the presence of retrogradely-transported Cre and systemic clozapine-n-oxide (CNO) administration causes mCherry-labelled cells to depolarise with temporal and anatomical specificity. The ABA protocol involves free access to running wheels and time-limited (90 min) access to food, with daily i.p. injections of CNO or saline at the onset of the feeding period.
CNO activates DREADD-expressing, VTA neurons as evidenced by colocalisation with elevated levels of Fos protein, a marker of neuronal activation. Importantly, excitation of this pathway with CNO attenuates the rapid weight loss associated with ABA with a profound effect on survival [$\chi^2(1)=9.95$, $p=0.002$]. The contribution of energy expenditure to body weight maintenance during activation is unclear. These results will inform the neurobiological underpinnings of AN, and provide insight into the mechanisms of reward circuitry relevant to feeding and weight loss.

Poster 120 – Monday 5th December

***MAPT* MUTATIONS AND SPORADIC FRONTOTEMPORAL TAUOPATHIES: LOST IN TRANSLATION?**

Dr Shelley Forrest[1], Professor Jillian Kril[1], Dr Claire Stevens[2], Associate Professor John Kwok[3,4,5], Dr Marianne Hallupp[3,5], Ms Sahar Latheef[3], Dr Woojin Kim[3,4,5], Dr Yue Huang[3,4], Ms Ciara McGinley[1], Ms Hellen Werka[1], Ms Heather McCann[3], Dr Andrew McGeachie[3,4], Professor Matthew Kiernan[5], Professor Jürgen Götz[6], Professor Maria Grazia Spillantini[7], Professor John Hodges[3,4,5], Professor Lars Ittner[2,3], Professor Glenda Halliday[3,4,5]

[1]*University Of Sydney,* [2]*Dementia Research Unit, School of Medical Sciences, University of New South Wales, ,* [3]*Neuroscience Research Australia,* [4]*School of Medical Sciences, University of New South Wales,* [5]*Brain and Mind Centre, The University of Sydney,* [6]*Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland,* [7]*Department of Clinical Neurosciences, University of Cambridge*

Genetic and familial forms of neurodegenerative disorders have provided important insights into the pathogenesis of many sporadic neurodegenerative disorders. In contrast to frontotemporal lobar degeneration (FTLD) with TDP-43-immunopositive inclusions, FTLD patients with mutations in the microtubule associated protein tau (*MAPT*) gene are considered independently of sporadic FTLD with tau-immunopositive inclusions (FTLD-tau) in neuropathological diagnostic criteria of which four subtypes are recognised: Pick's disease (PiD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and globular glial tauopathy (GGT). All FTLD cases with a *MAPT* mutation in the Sydney-Cambridge cohorts were screened for differentiating features used to diagnose FTLD-tau subtypes to determine whether categorical separation of *MAPT* mutations from sporadic FTLD-tau is valid. Using multiple tau antibodies, the type and distribution

of neuropathological features were compared between 11 cases with a *MAPT* mutation (including 3 S305S mutation siblings) and 16 sporadic FTLD-tau cases (PiD=4, CBD=4, PSP=4, GGT=4). Cases with a *MAPT* mutation were younger at age of symptom onset (57±5 vs. 70±6 years) but showed no difference in disease duration. Neuropathological phenotype associated with *MAPT* mutations varied between and within families with the same mutation. Each case with a *MAPT* mutation had similar neuropathological features to one of the sporadic FTLD-tau subtypes and could be classified into a comparable diagnostic subtype: PiD (Lys257Thr), CBD (S305S, IVS10+16, R406W), PSP (S305S), GGT (P301L, IVS10+16). This study demonstrates similarities in neuropathology between genetic and sporadic forms of FTLD-tau indicating that *MAPT* mutation cases should be considered as familial forms of FTLD-tau on a disease continuum.

Poster 121 – Tuesday 6th December

THE MICROTUBULE-STABILISING DRUG EPOTHILONE D ALTERS SPECIFIC ASPECTS OF NEURONAL PLASTICITY FOLLOWING MILD TRAUMATIC BRAIN INJURY IN ADULT MICE

Jyoti Chuckowree[1], Clara Lee[1], Mariana Brizuela[1], Tracey Dickson[1]

[1]Menzies Institute For Medical Research

Mild traumatic brain injury (TBI), or concussion, results from an external force causing a temporary loss or alteration of consciousness. This relatively subtle perturbation may evoke widespread damage to neural circuitry, culminating in ongoing neurological impairment. Damage to the neuronal cytoskeleton, particularly dissolution of microtubules, has been identified as a major contributor toward impaired neuronal function following brain injury. Here, we investigated the potential benefits of the microtubule stabilising agent, Epothilone D (EpoD), in a mouse model of mild TBI. Mild lateral TBI was generated using the fluid percussion device in adult male Thy1-YFPH mice. Mild injury was confirmed by transient apnoea (<15secs) and delayed righting times (2-6mins), as well as a lack of overt neuronal loss and absence of cortical cavitation, relative to sham-operated animals. Following brain injury or sham-operation mice were treated with EpoD (2mg/kg, ip) or vehicle (DMSO) and perfused 7 days thereafter. Diffuse axonal injury and dendritic spine loss were hallmark features of the post-injury sequelae in Thy1-YFPH expressing neurons. Notably, post-injury EpoD treatment resulted in significant (p<0.05) preservation of axons in the injured internal capsule. With regard to synaptic alterations, EpoD treatment significantly (p<0.05) increased dendritic spine density (p<0.05), while spine length was significantly decreased (p<0.05). Moreover, we recorded a significant (p<0.05) shift in spine morphology, whereby the proportion of mushroom spines was decreased with a concomitant increase in thin spines. Collectively, these findings indicate that stabilisation of microtubules following mild brain injury may combat key pathological alterations including synapse loss and axonal degeneration.

Poster 122 – Monday 5th December

EFFECTS OF iTBS ON MOLECULAR MARKERS OF NEURAL PLASTICITY IN THE MOTOR CORTEX: FINDINGS FROM RODENT-COIL, IN VIVO ANIMAL MODEL

Barbora Fulopova[1,2], Dr William Bennett[1], Dr Jessica Collins[1], Alexander Tang[4], Dr Michael Garry[3], Dr Mark Hinder[3], Professor Jeffery Summers[3,5], Dr Jennifer Rodger[4], Professor James Vickers[1], Dr Alison Canty[1]

[1]Wicking Dementia Research and Education Centre, University of Tasmania, [2]School of Medicine, Faculty of Health, University of Tasmania, [3]Human Motor Control Lab, School of Medicine, University of Tasmania, [4]Department of Experimental and Regenerative Neurosciences, School of Animal Biology, University of Western Australia, [5]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University

Delivery of intermittent theta burst stimulation (iTBS) modulates cortical excitability, and has been reported to induce neural plasticity when delivered over the primary motor cortex. Despite widespread clinical and non-clinical use of iTBS in humans, neural responses to iTBS remain largely unknown. Results from studies using a human sized coil to stimulate organotypic hippocampal cultures suggest involvement of LTP/LTD-like events over 1-6 hours post-stimulation. A smaller, rodent-sized coil, allows for *in vivo* administration of iTBS protocols to rodents in a fashion that more closely resembles administration of iTBS in humans. Using F-actin as a marker of structural plasticity associated with LTP/LTD-like changes, 15 wild type mice received iTBS, or a sham stimulation, over primary motor cortex, and the brains were obtained 2 hours after the treatment for immunohistochemical analysis. Fluorescence intensity of F-actin staining was on average 10% lower in the iTBS group compared to sham, however, this difference did not reach statistical significance. Changes in F-actin were also investigated in animals that demonstrated increased forelimb reaching accuracy over 10 days in a motor learning task, where iTBS was shown to modulate reaching accuracy. Changes in expression of F-actin were compared between four groups (priming iTBS delivered before skilled reaching, consolidating iTBS delivered immediately after skilled reaching, motor learning only, handling control). Preliminary analysis suggests no remarkable differences in the levels of F-actin between these treatment groups. These results indicate possible transient LTD-like effects of iTBS upon stimulation, that do not persist over time.

Poster 123 – Tuesday 6th December

ACTIVATION OF TOLL-LIKE RECEPTOR 2 INCREASES ALPHA-SYNUCLEIN LEVELS IN NEURONAL CELLS

Jianqun Gao[1,2], GAYATHRI PERERA[1], Doctor NICOLAS DZAMKO[1,2], Professor Glenda Halliday[1,2]

[1]Neuroscience Research Australia, [2]School of Medical Sciences, University of NSW, Australia

Parkinson's disease (PD) is a progressive neurodegenerative disorder with the formation and development of Lewy bodies (LBs) and Lewy neurites (LNs) as its pathological characteristics. The most prominent and well-studied component of LBs and LNs is □-synuclein, which is thought to propagate through PD brain in a prion-like manner contributing to neural dysfunction. New evidence suggests that toll-like receptor 2 (TLR2), a member of the innate immune pattern recognition receptor family, may contribute to the spread of □-synuclein in PD brain [1]. In this study we used differentiated SHSY5Y cells, and primary IPS-derived neural progenitor cells and treated them with PAM3CSK4, a potent agonist for TLR2. Over a one-week period we observed a significant increase in levels of endogenous □-synuclein by both imaging and immunoblotting. The increase in □-synuclein was associated with an increase in the selective autophagy marker p62/SQSTM1, suggesting □-synuclein may be increased due to impaired autophagy-lysosomal clearance. Moreover, the TLR2-stimulated accumulation of □-synuclein could be prevented by promoting autophagy with rapamycin. Finally we found that a number of small molecule inhibitors that target the TLR2 pathway could also prevent the accumulation of □-synuclein in neural cells. These results suggested that targeting TLR2 and/or the AKT-mTORC1 signaling pathway might be a potential therapeutic option for preventing α-synuclein accumulation in PD.

1 Kim, C., E. Rockenstein, B. Spencer, H.K. Kim, et al., *Antagonizing Neuronal Toll-like Receptor 2 Prevents Synucleinopathy by Activating Autophagy.* Cell Rep, 2015. **13**(4): p. 771-82.

Poster 124 – Monday 5th December

INVESTIGATING THE PATHOLOGICAL MECHANISMS UNDERLYING RAB39B-MEDIATED PARKINSON'S DISEASE USING NOVEL CELL MODELS.

Ms Y Gao[1,2], Dr GR Wilson[1,2], Dr SEM Stephenson[1,2], Ms M Giannandrea[3,4], Dr P D'Adamo[3,4], Dr M Dottori[5], Assoc. Prof. PJ Lockhart[1,2]
[1]Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, [2]Department of Paediatrics University of Melbourne, [3]Dulbecco Telethon Institute at Division of Neuroscience, San Raffaele Scientific Institute, [4]Hoffmann- La Roche AG, pRED Pharma Research & Early Development, NORD Neuroscience, [5]Centre for Neural Engineering University of Melbourne

Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra and the presence of α-synuclein (αSN) aggregates in surviving dopaminergic neurons. We recently demonstrated that mutations in Ras Analog in Brain 39B (*RAB39B*) cause X-linked early onset PD. Our aim is to generate novel models dysregulated for *RAB39B* expression to investigate the encoded protein's role in the initiation and development of PD. We generated cell models with *Rab39b* knockdown in a mouse neural progenitor cell line (P19) and primary hippocampal neurons (E18) via lentiviral transduction of shRNA targeted for *Rab39b*. Western blot and immunofluorescence analysis showed significant reductions in steady state RAB39B levels (40-80%, $p<0.05$), and significant reductions in steady state αSN levels (40-50%, $p<0.05$) in *Rab39b* knockdown cell lines compared to controls. We also generated pluripotent stem cell models with *RAB39B* deletion. Induced pluripotent stem cells (iPSC) were generated from *RAB39B* deletion patient fibroblasts using Sendai virus carrying standard reprogramming factors. In addition, we generated isogenic lines with the deletion of *RAB39B* in a human embryonic stem cell (hESC) line, MEL1, via CRISPR/Cas9. *RAB39B* deletion was confirmed in the iPSC and hESC lines by PCR analysis and Sanger sequencing, and the stem cells display high expression of pluripotency markers and were able to differentiate into different germ layers. In conclusion, our study suggests the pathological mechanisms underlying *RAB39B*–mediated PD involves perturbation of aSN regulation and homeostasis. This will be investigated further in neuron models differentiated from pluripotent stem cells.

Poster 125 – Tuesday 6th December

INVESTIGATING THE SUBCELLULAR LOCALIZATION OF HUMAN TYROSINE HYDROXYLASE ISOFORMS

Mr Pedro Garcia Sobrinho[1], Ms Alice Kunzler[2], Ms Tenele Smith[1], Dr Gabrielle Briggs[1], Dr Matthew Dun[1], Dr Trisha Al-mazi[1], Emeritus Professor Peter R Dunkley[1], Dr Associate Professor Philip W Dickson[1]

[1]The University Of Newcastle Australia, [2]Federal University of Rio Grande do Sul, Porto Alegre

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of the catecholamines. Humans are unique in that they have four different isoforms. There is very little information on the different roles of these isoforms and we hypothesised that they may show differential subcellular localisation. To examine the subcellular localisation of TH, the SH-SY5Y cell line was transfected with the human TH isoform 1 (hTH1) or isoform 4 (hTH4), the two isoforms with the greatest difference in size. Subcellular distribution was determined under basal and muscarine stimulated conditions. TH was found primarily in the cytosol with around 20% in the membrane-associated fraction and a small amount in the nuclear fraction. There was no difference in the distribution of the two isoforms in these fractions with respect to total TH protein and pSer19 TH under basal conditions. In contrast, the level of pSer40 hTH4 was around two fold higher in the membrane-associated fraction than pSer40 hTH1 ($p< 0.001$). After muscarine stimulation the level of total hTH4 protein was significantly lower than hTH1 in the cytosolic fraction ($p < 0.001$) and there was a corresponding increase in the level of hTH4 in the nuclear fraction, where hTH1 was not detectable. The level of pSer19 hTH4 was significantly lower than pSer19 hTH1 in the membrane fraction after muscarine stimulation ($p<0.01$), whereas there were no differences in the two isoforms in relation to pSer40 distribution. We are currently analysing differential protein binding to these two isoforms to understand the basis of this differential distribution.

Poster 126 – Monday 5th December

OLIGODENDROCYTE PROGENITOR CELL PROCESS DYNAMICS AND MOTILITY ARE REGULATED BY STORE OPERATED CALCIUM ENTRY IN VITRO.

Dr Robert Gasperini[1,2], A/Prof Lisa Foa[2], Dr Kaylene Young[1]

[1]Menzies Institute for Medical Research, [2]School of Medicine, UTAS

During development, oligodendrocyte progenitor cells (OPCs) migrate and populate the CNS before differentiating into myelin-producing oligodendrocytes (OLs). This process continues in adulthood, particularly in response to demyelinating disease, where OL replacement is coordinated by OPC proliferation and migration. The molecular mechanisms that regulate OPC motility are yet to be fully elucidated. Cytosolic calcium (Ca^{++}) is a crucial regulator of migration and motility in many cells during development. Calcium dynamics during motility are sustained by Ca^{++} from multiple sources including internal Ca^{++} stores such as the endoplasmic reticulum in a process called store operated Ca^{++} entry (SOCE). In this study we asked if the SOCE regulator, stromal interacting molecule (STIM) was necessary for OPC motility. STIM1 and STIM2 protein isoforms are signalling partners that mediate SOCE and basal Ca^{++}. Using an siRNA approach, we show that STIM2 (63.8+/-4.7% of controls) but not STIM1 (97.2+/-2.1% of controls) is necessary for maintaining basal Ca^{++} levels in OPCs. Using high-resolution ratiometric Ca^{++} imaging, we also determined that OPC processes and lamellapodia are highly motile with Ca^{++} levels correlated to protrusive activity and Ca^{++} transient frequency. Additionally, after store depletion SOCE was significantly increased in OPC with motile lamellapodia compared to stable or non-motile cells (protrusive lamellapodia 1274+/-62 nM Ca^{++} compared to stable lamellapodia 311+/-32 nM Ca^{++}). These data suggest that STIM proteins are relevant targets to study the role of Ca^{++} in myelinogenesis in the developing and adult CNS

Poster 127 – Tuesday 6th December

NANOWIRE ARRAYS GUIDE THE FORMATION OF NEURONAL CIRCUITS

Vini Gautam[1], Shagufta Naureen[2], Dr Naeem Shahid[2], Ms Qian Gao[2], Ms Yi Wang[3], Dr David Nisbet[3], Chennupati Jagadish[2], Vincent Riccardo Daria[1]

[1]John Curtin School of Medical Research, Australian National University, [2]Research School of Physics and Engineering, Australian National University, [3]Research School of Engineering, Australian National University

Purpose: Engineering neuronal networks on artificial substrates could provide a better understanding of the role of physico-chemical cues leading to circuit formation and establish design parameters to build scaffolds for neuroprosthetics. Here, we use vertically aligned Indium Phosphide (InP) nanowire (NW) arrays to engineer the formation of functional neuronal networks *in vitro*. **Methods:** The NW arrays were fabricated using electron beam lithography, followed by a combination of dry and wet etching. The pitch of these NW arrays was varied from 0.5 - 5 □m, and the nanowire diameter was varied from 150 – 750 nm. Hippocampal cells were isolated from the brains of P0-P2 rats (Wistar). The cells were suspended in Dulbecco's modified Earl's medium with relevant supplements and plated on the NW substrates and on a glass coverslip for control. The neurite growth was monitored using immunocytochemistry while cellular dynamics was observed using a fluorogenic calcium indicator (Cal-520AM). **Results:** Our studies show that isotropic arrangement of NWs results in a highly directional growth of neurites tracing the NW patterns. The neurites first align along the NWs and eventually form a dense and ordered network. This effect is observed for a NW pitch < 2µm. Calcium activity shows synchronized neuronal transients in the neuronal network growing on the NWs compared to that on glass. **Conclusion:** We show that NW arrays provide topographical cues that guide the growth of neurons forming functional circuits with highly correlated cellular activity. Understanding these nano-topographical cues could provide fresh insights to theories on neuronal circuit formation.

Poster 128 – Monday 5th December

CONTROL OF A SPECIFIC PROTEOLYSIS EVENT USING LIGHT

Dr David Gell[1], Dr John Lin[1]

[1]University of Tasmania

The ability to fluorescently label synapses that are activated during a behavioural task would provide a powerful tool to study the structural basis of learning and memory. One way to highlight a sub-set of synapses is to express the complementary fragments of a split fluorescent protein (split-FP) on the pre- and post-synaptic membranes of two genetically defined cell types (Feinberg et al. 2008, *Neuron*). As an extension of this strategy, one component of the split-FP was delivered to the synapse in synaptic vesicles, in order to enhance fluorescent labelling of more active synapses in *Drosophila* (Macpherson et al. 2015, *Nature Communications*). However, to identify neuronal circuits involved in behaviour, it is important to restrict synaptic labelling to the time window in which a task is performed. Our strategy to achieve a temporal window of synaptic labelling is to make fluorescence complementation dependent upon synaptic activity *and* simultaneous illumination of the synapse with light. In pre-existing designs, split-FPs were anchored to the pre- and post-synaptic membranes in such a way as to allow complementation across the synaptic cleft. We aim to present one split-FP in a conformation that cannot span the synaptic cleft and, instead, permit complementation by proteolytic cleavage of the split-FP away from its membrane anchor. To this end, we describe six-fold activation of a specific protease cleavage using light. We envisage delivering split-FP and its cognate site-specific protease into the synapse via synaptic vesicle fusion, thus making fluorescence complementation dependent on both synaptic activity and light-stimulated protease cleavage.

Poster 129 – Tuesday 6th December

METALLATION ALTERATIONS OF SUPEROXIDE DISMUTASE 1 AND METALLOTHIONEIN-II IN THE PARKINSON'S DISEASE BRAIN

Sian Genoud[1], Benjamin Trist[1], Dr Dominic Hare[2], Dr Blaine Roberts[3], Associate Professor Kay Double[1]

[1]University of Sydney, [2]University of Technology, Sydney and the Florey Institute of Neuroscience and Mental Health, University of Melbourne, [3]The Florey Institute of Neuroscience and Mental health, University of Melbourne

Decreased copper (Cu) is a feature of vulnerable regions in the Parkinson's disease (PD) brain and we have recently demonstrated that the antioxidant cuproprotein superoxide dismutase 1 (SOD1) is dysfunctional in these Cu-deficient regions. Cu-deficient SOD1 is associated with motor neuron death in another neurodegenerative disorder, Amyotrophic lateral Sclerosis (ALS), thus we propose that Cu-deficient SOD1 may similarly contribute to neuronal vulnerability in PD. The current study employed degenerating and non-degenerating regions of the PD brain (n=8) and age-matched controls (n=8), to perform a bulk metal analysis and global metalloproteomic analysis through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Size Exclusion Chromatography hyphenated with ICP-MS respectively. Total Cu in the degenerating substantia nigra was reduced by 54% (p=0.03) in PD. The soluble tissue fraction ratio of Cu to zinc (Zn) was significantly altered in the substantia nigra in PD (41% decrease; p=0.03); this change was also observed for metal-associated proteins alone in this brain region (51% decrease; p=0.006). The alterations in Cu- and Zn-associated proteins were confined to two distinct molecular masses, corresponding to the protein standards for SOD1 and metallothionein-II, suggesting metallation of these proteins is altered. As these changes were not observed in the non-degenerating regions, the current data suggest that reduced Cu-binding, and subsequent dysregulation of cuproproteins, may contribute to neuronal vulnerability in PD.

Poster 130 – Monday 5th December

LONG-TERM IMAGING IN VIVO OF SPINE DYNAMICS ON A PUTATIVE VIP DISINHIBITORY NEURONAL SUBCLASS

Christina Georgiou[1,2], Daniela Sahlender[3], Vassilis Kehayas[1,2], Graham K. Knott[3], Anthony Holtmaat[1]

[1]Department of Basic Neurosciences and Geneva Neuroscience Center, University of Geneva, Switzerland, [2]Lemanic Neuroscience Doctoral School, Switzerland, [3]Ecole Polytechnique Fédérale de Lausanne

In supragranular layers of the cerebral cortex, excitatory pyramidal cells are inhibited by parvalbumin (PV)- and somatostatin (SST)-expressing interneurons, which in turn are inhibited by vasoactive intestinal peptide (VIP)-expressing cells. VIP-cells receive long-range excitatory inputs, which may thereby disinhibit pyramidal cells and play an important role in associative cortical learning mechanisms.
Here, we used VIP-Cre transgenic mice in combination with Cre-dependent AAV-GFP vectors to label and image VIP cells in superficial cortical layers *in vivo*. We found that the dendrites of a subpopulation of the labeled cells, characterized by a multipolar morphology (4.7±0.95 dendrites/cell), bear spines (0.79±0.08 spines/µm). Longitudinal 2-photon laser scanning microscopy *in vivo* indicates that these appear and disappear under baseline conditions (0.25±0.03 per 4 days). Their survival fractions are lower as compared to what has been reported for pyramidal cells. Using survival analysis (Cox Proportional Hazards Regression), we estimate that spines on VIP neurons have a higher instantaneous failure rate as compared to spines on pyramidal cells (beta=0.69, p<0.001). Electron microscopy shows that each spine has an excitatory synapse, but their ultrastructure is distinct from those of pyramidal neurons. Using trans-synaptic tracing we confirmed that their inputs include various long-range projections.
Together, our data suggest that a group of superficial cortical VIP cells receive excitatory inputs on structurally dynamic spines. Their relatively high degree of plasticity has the potential to regulate the strength and the source of their excitation, and thereby dynamically shape network disinhibition.

Poster 131 – Tuesday 6th December

EXPERIMENTAL USE OF INVASIVE BRAIN TECHNOLOGIES FOR TREATMENT OF PSYCHIATRIC CONDITIONS: WHAT CAN WE LEARN FROM THE PAST?

PhD Frederic Gilbert[1], John Noel M. Viana[1]

[1]UTAS

The history of neuroscience accounts for many changes in the ways medicine has explained psychiatric condition aetiologies; and consequently, has modified the ways some disorders have been treated. Recently, there has been growing interest in using invasive brain surgery, involving implantable devices to alleviate the symptoms of patients suffering from different types of neurological and psychiatric conditions (i.e. Treatment Resistance Depression, Obsessive Compulsive disorder, Alzheimer, etc.), even though the origin of these conditions is still far from being fully understood. The purpose of this presentation is to explore the new ethical issues of using novel invasive and implantable technologies to treat psychiatric conditions.

Poster 132 – Monday 5th December

EXPRESSION CHANGES OF CHLORIDE INTERACTING PROTEIN 1 DUE TO THERAPEUTIC HYPOTHERMIA IN A MODEL OF NEONATAL HYPOXIC-ISCHAEMIC ENCEPHALOPATHY

Miss Kate Goasdoue[1], Ms Stephanie Miller[1], Ms Leah Billing[1], Professor Paul Colditz[1], Dr Tracey Bjorkman[1]

[1]Perinatal Research Centre, UQ Centre for Clinical Research, The University of Queensland

Neonatal hypoxia ischaemia (HI) occurs in approximately 1-5 per 1000 live term births and may result in long term neurodevelopmental disorders. The only currently available treatment is therapeutic hypothermia – which is thought to act upon multiple pathways of cell death and neurological injury following HI, however, exact mechanisms of neuroprotection are poorly understood. The objective of this study was to investigate changes to protein expression levels of the cation chloride co-transporter family in a piglet model of HI treated with therapeutic hypothermia. Piglets (n=16) were administered an acute HI insult of approximately 30 minutes and then were treated with moderate hypothermia (5°C reduction in body temperature for 24h) or maintained at normal body temperature. Piglets were survived to 72h when MRI measures were performed to assess injury severity. Protein expression levels of chloride-interacting protein 1 (CIP1) were analysed by western blot. Hypothermia animals had significantly less brain injury than normothermic animals as determined by lactate to N-acetylaspartate ratio ($p<0.05$). Signficantly higher levels of CIP1 were found in the hypothermic group in the parietal cortex and hippocampus ($p<0.05$). Higher levels of CIP1 were associated with better neurological outcomes. Changes to protein expression levels such as CIP1 may be one mechanism through which therapeutic hypothermia is acting to promote neuroprotection.

Poster 133 – Tuesday 6th December

RAPID MODULATION OF NEURONAL VOLTAGE-GATED CALCIUM CHANNELS BY VITAMIN D DURING DEVELOPMENT

Doctor Helen Gooch[1], Doctor Xiaoying Cui[1], Doctor Victor Anggono[1], Associate Professor Thomas Burne[1], Professor Darryl Eyles[1], Professor Pankaj Sah[1], Professor John McGrath[1]

[1]Queensland Brain Institute

Aim:
The secosteroid vitamin D [$1,25(OH)_2D_3$] is known to drive non-genomic effects in peripheral tissues, principally the rapid modulation of L-type voltage-gated calcium channels (VGCC). However, its non-genomic effects within the brain remain unexplored. Since developmental vitamin D deficiency is a risk factor for schizophrenia, and common variants in L-VGCC subunits are associated with neuropsychiatric disorders, we investigated the non-genomic effects of $1,25(OH)_2D_3$ on VGCCs in the developing brain.
Methods:
All methods performed on coronal slices from p8-12 BALB/C mice. Wide-field Ca^{2+} imaging (CAL-520) was time-locked to field stimulation using parallel platinum electrodes. Nucleated patch recordings utilised Ba^{2+} as the charge carrier, with VGCC currents isolated pharmacologically.
Results:
Using nucleated patch (n=3/14) and wide-field Ca^{2+} imaging, $1,25(OH)_2D_3$ (0.1 nM) was shown to rapidly increase somatic Ca^{2+} levels in a subset of neurons, termed ***vitamin D responsive neurons*** (***VDRNs***). Additionally, wide-field Ca^{2+} imaging revealed that $1,25(OH)_2D_3$ induced several other heterogeneous responses, including ΔF/F *increases* that were acute or delayed, along with delayed onset *decreases* in ΔF/F (n=127 cells, n=19 slices).
Conclusion:
These findings demonstrate that physiological levels of $1,25(OH)_2D_3$ rapidly modulate VGCCs in a subset of PFC neurons during development, with putative consequences for neuronal maturation and network performance. Future experiments will investigate the molecular mechanism underlying this effect, along with VDRN identity and distribution

Poster 134 – Monday 5th December

THE NLRP3 INFLAMMASOME DRIVES SYNUCLEIN PATHOLOGY AND PROGRESSIVE DOPAMINERGIC DEGENERATION IN PARKINSON'S DISEASE

Dr Richard Gordon[1], Eduardo Albornoz A[1], Daniel Christie C[1], Monica Langley R[2], Vinod Kumar[1], Dr Susanna Manotovani[1], Dr Avril Robertson AB[1], Dr Mark Butler S[1], Professor Dominic Rowe B[4], Professor Luke O'Neill A[3], Professor Kanthasamy AG[2], Dr Kate Schroder[1], Professor Alister Cooper M[1], A/Prof. Martin Woodruff T[1]

[1]The University Of Queensland, Australia, [2]Iowa State University, [3]School of Biochemistry and Immunology, [4]Macquarie University

Parkinson's disease (PD) pathology is characterized by a profound loss of nigral dopaminergic neurons that is accompanied by chronic neuroinflammation and extensive α-synuclein inclusions in the form of Lewy-bodies. Fibrillar synuclein has recently been shown to be the major neurotoxic species in PD, mediating cell-to-cell transmission and progressive neuropathology. However, the mechanisms by synuclein pathology and spread contributes to dopaminergic degeneration is unclear. Chronic activation of the NLRP3 inflammasome in the CNS by insoluble protein aggregates, is emerging as a major pathological mechanism that can drive progressive neurodegeneration. Herein, we demonstrate that activation of the microglial NLRP3 inflammasome is a common pathway triggered by both fibrillar synuclein and by dopaminergic degeneration in the absence of synuclein aggregates. Key hallmarks of inflammasome activation including cleaved caspase-1 p20, and ASC upregulation are evident in the substantia nigra of PD patients. Similarly, we also found extensive NLRP3 inflammasome in

multiple pre-clinical mouse models of PD. Our mechanistic studies with primary microglia demonstrate that fibrillar Syn activates the NLRP3 inflammasome with delayed kinetics compared to canonical NLRP3 agonists. Crucially, we demonstrate that the potent NLRP3 inhibitor, MCC950, is active in the central nervous system following oral dosing, and can effectively block inflammasome activation and neuropathology in PD models. Significantly, chronic daily oral dosing of MCC950 effectively protected against motor deficits and nigrostriatal dopaminergic degeneration induced by synuclein fibrils in the pre-formed fibril model of synuclein pathology. Collectively, these findings suggest that the microglial NLRP3 inflammasome pathway could be a sustained source of neuroinflammation that drives PD pathology, and highlights microglial NLRP3 as a novel therapeutic target for PD.

Poster 135 – Tuesday 6th December

FLUORESCENT INTRA-BODY LOCALIZATION MICROSCOPY (FILM): A NOVEL METHOD FOR SUPER-RESOLUTION OF GFP-TAGGED INTRACELLULAR PROTEINS

Ms Rachel S. Gormal[1], Mr Ravikiran Kasula[1], Mr Adekunle D. Bademosi[1], Dr Pranesh Padmanabhan[2], Mr James Rae[3], Mr Charles Ferguson[3], Dr Brett M. Collins[3], Dr Geoffrey J. Goodhill[4], Dr Robert G. Parton[3], Dr Frederic A. Meunier[1]

[1]The University Of Queensland, Queensland Brain Institute, Clem Jones Centre for Ageing Dementia Research, Brisbane, [2]The University of Queensland, Queensland Brain Institute, [3]The University of Queensland, Institute for Molecular Bioscience, [4]The University of Queensland, School of Mathematics and Physics

Novel super-resolution microscopy techniques have provided unprecedented details of biological structures and processes by breaking the Abbe law of diffraction. The utilization of these techniques to their full potential generally relies on novel reagents, large highly specialised constructs and sophisticated sample preparation techniques. Herein, we describe the utilization of single chain 'intra-bodies' that can be easily applied to perform single molecule imaging of any intracellular GFP-tagged protein. In contrast to traditional antibodies, small camelid 'nanobodies' with their small size, limited aggregative propensity and the current versatility of their application are thus highly suitable for this application. Here we show that expression of nanobodies targeted against GFP and tagged with a photoconvertible mEOS2 allows super-resolution imaging of GFP-tagged proteins in live and fixed cells, with unprecedented accuracy. Neurosecretory PC12 cells expressing phosphatidylinositol(4,5)bisphosphate probe PH-PLCdelta-mEOS2 were compared with cells expressing both PH-PLCdelta-GFP and anti-GFP nanobody-mEos2. In fixed cells, the PIP2 nanoclusters detected by TIRF-PALM microscopy using the two probes had the same size and density. In live cells, the mobility of the two probes detected by sptPALM was also identical. Combining Nanobodies with an Apex tag allowed us to perform 3D Electron Microscopy on PIP2 nanoclusters. Fluorescent intra-body localization Microscopy (FiLM), therefore enables GFP-tagged constructs to be localised by super-resolution microscopy, reducing cloning and reagent requirements significantly. In principle, this method could be extended to other specific intra-bodies to perform super-resolution imaging of endogenous proteins.

Poster 136 – Monday 5th December

AN OPTOGENETIC CHARACTERIZATION OF PRESYNAPTIC INHIBITION IN DORSAL HORN MICROCIRCUITS

Mr Mark Gradwell[1], Prof. Robert Callister[1], Dr. David Hughes[2], Assoc. Prof Brett Graham[1]

[1]University Of Newcastle, [2]University of Glasgow

Two forms of inhibition; postsynaptic (axo-dendritic) and presynaptic (axo-axonic) play a critical role in spinal sensory coding. We have recently characterised a population of inhibitory interneuron (Parvalbumin-positive interneurons PVINs) that provide presynaptic inhibition onto myelinated afferent terminals within the dorsal horn (Hughes, 2012 J Physiol 16:3927). Here, we investigate the connectivity of PVINs and the dorsal horn circuitry they regulate through presynaptic inhibition. Briefly, parasagittal lumbar spinal cord slices were prepared from transgenic mice expressing Channelrhodopsin-2 in PV^{+}INs. Whole cell patch clamp recordings were made from unidentified (PV-negative) neurons during brief whole-field photostimulation (488nm, 1ms). During inhibitory recordings (Cs-Cl internal), photostimulation evoked polysynaptic mixed GABA/Gly postsynaptic currents in 87% of PV-negative recordings (26/30). Addition of Strychnine increased peak amplitude by 38%, whereas addition of bicuculline reduced peak amplitude by 94%. Similarly addition of the CNQX abolished polysynaptic IPSCs. During excitatory recordings (K-gluc internal) photostimulation evoked short latency excitatory postsynaptic currents (EPSCs) in 42% of recordings (83/200), presumably arising from a small population of excitatory PVINs. In other recordings (32%, 65/200), photostimulation evoked longer latency EPSCs that could be blocked by CNQX, but also bicuculline. A subset of these neurons with specific firing properties fired action potentials following photostimulation. Together these findings imply a small population of inhibitory interneurons exist, that are postsynaptic to specialised PVIN regulated afferent input. It is likely this population is part of a critical microcircuit that segregates tactile and nociceptive inputs within the dorsal horn of the spinal cord.

Poster 137 – Tuesday 6th December

HETEROMERIC BETA SUBUNIT CONTAINING GLYCINE RECEPTORS MEDIATE SYNAPTIC AND TONIC INHIBITION IN PARVALBUMIN POSITIVE SPINAL DORSAL HORN NEURONS

Mr Mark Gradwell[1], Prof Robert Callister[1], Dr David Hughes[2], **A.prof Brett Graham[1]**

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, [2]Institute of Neuroscience Psychology, University of Glasgow

The spinal dorsal horn contains a heterogenous population of interneurons and is a key site for nociceptive processing. Inhibition is fundamental to this processing and is predominantly mediated by GABA and glycine. Previous work has shown that both GABA and glycine can activate synaptically clustered, or extrasynaptically distributed receptors, corresponding to phasic and tonic forms of inhibition. Work in a variety of CNS regions, including the dorsal horn, has established that distinct pools of GABA receptors are targeted to synaptic and extra synaptic locations based on subunit composition. In contrast, the importance of subunit composition for targeting glycine receptors is unclear. To address this issue we made targeted patch-clamp recordings in spinal cord slices from transgenic mice (n=9), expressing enhanced green fluorescent protein (eGFP) in parvalbumin positive interneurons, which exhibit substantial synaptic and tonic glycinergic inhibition. For synaptic receptors, miniature inhibitory post-synaptic currents (mIPSCs) were recorded and peak scaled non-stationary noise analysis estimated a mean single channel conductance of 36±3 pS (n=20). In addition, picrotoxin did not change mIPSC frequency, amplitude, or time course (n=8). Together, these data are consistent with heteromeric beta subunit-containing glycine receptors. For extrasynaptic receptors, tonic currents were also picrotoxin insensitive but abolished by bath-applied strychnine (n=9). Non-stationary noise analysis during the strychnine block estimated a mean single channel conductance of 29±1 pS (n=19). These data also suggest heteromeric beta subunit-containing glycine receptor composition. Thus, unlike the GABAergic system, glycine receptor subunit composition does not appear to be a determinant of synaptic versus extrasynaptic localization.

Poster 138 – Monday 5th December

PROFILING A BIOCHEMICAL MODEL OF PRESYNAPTIC DEPRESSION OF NEUROTRANSMITTER RELEASE USING PHOSPHOPROTEOMICS

Dr Kasper Engholm-Keller[1], Prof. Phillip Robinson[1], **Dr Mark Graham[1]**

[1]Children's Medical Research Institute, The University of Sydney

A strong or high frequency stimulation of presynaptic terminals can cause a short term depression of neurotransmitter release. The cause of this depression will be in part due to vesicle depletion but other mechanisms are involved. Protein phosphorylation and dephosphorylation is a crucial fast signalling mechanism following depolarisation of presynaptic nerve terminals. Depolarisation causes a calcium influx, which activates a signalling cascade mediated by protein kinases and phosphatases. Short and long term changes to the level of phosphorylation on presynaptic proteins is expected to modulate neurotransmitter release. We have used strong chemical depolarisation of isolated presynaptic nerve terminals to profile presynaptic signalling, which included a time course of "repolarisation". Quantitative analysis of greater than 1900 significantly changing phosphorylation sites has allowed the first detailed assessment of the signalling resulting from this stimulus. The signalling has been correlated with a depression in glutamate release. We have shown that the type and level of stimulus determines the presynaptic response and have identified regulators of the synaptic vesicle cycle and other biological processes as targets for activity dependent phospho-signalling.

Poster 139 – Tuesday 6th December

IDENTIFICATION OF DIFFERENT TYPES OF SPINAL AFFERENT ENDINGS IN THE URINARY BLADDER USING ANTEROGRADE TRACING FROM DORSAL ROOT GANGLIA IN VIVO.

Miss Sarah Greenhalgh[1], Miss Melinda Kyloh[1], Professor Vladimir Zagorodnyuk[1], Professor Nicholas Spencer[1]

[1]Flinders University

Sensory stimuli within visceral organs are detected by spinal afferent neurons, whose cell bodies lie in dorsal root ganglia (DRG). In the urinary bladder, the nerve endings of spinal afferents that detect noxious (painful) and innocuous stimuli have never been identified due to a lack of techniques available. The aim of this study was to identify the different morphological types of spinal afferent nerve endings within the urinary bladder and determine their immunoreactivity to CGRP. We used a technique recently developed in our laboratory which selectively labels spinal afferent axons and their nerve endings. This involved injection of dextran biotin into L6-S3 DRG of 4 mice in vivo. Seven days post injection, mice were euthanized. Of all labelled nerve axons, only 1 ending (complex-type) was identified within the urothelium; the remaining 23 discrete endings ramified within the detrusor muscle. Within the detrusor muscle, three distinct types of endings were identified, consisting of complex-type (50%), simple-type (29%) and branching-type (21%) (N=4). Complex-type endings consist of multiple branching axons with no consistent alignment with any muscle fibres. Simple-type endings consist of single, non-branching axons that mostly ramified parallel to muscle fibres. Branching-type endings are analogous to vagal intramuscular arrays, and consist of multiple simple varicose endings that ramified parallel to each other. Of all identified nerve endings, most were CGRP-immunoreactive (75% of complex endings), (100% of simple endings) and (60% branching endings) were CGRP positive. This is the first identification of spinal afferent endings in the urinary bladder of a mammal.

Poster 140 – Monday 5th December

ALPHA-SYNUCLEIN TOXICITY IS ABOLISHED BY LIPROXSTATIN-1 THROUGH A FERROPTOSIS-INDEPENDENT MECHANISM

Stephanie J Guiney[1], Associate Professor Paul A Adlard[1], Professor Ashley I Bush[1], Associate Professor David I Finkelstein[1], Dr Scott Ayton[1]

[1]The Florey Institute Of Neuroscience And Mental Health

Background: There is good evidence that **a**lpha-synuclein (αsyn) and iron both contribute to neurotoxicity in Parkinson's disease (PD). However, it is unknown how they cause cell death, and whether the toxicity of αsyn and iron is synergistic or independent.
Objective: To investigate whether αsyn causes neurodegeneration by ferroptosis (a recently identified iron-dependent cell death pathway).
Methods: Toxicity of pre-formed fibril preparations of αsyn was assessed in multiple immortalised and primary culture cell lines (C57Bl/6/129sv mouse primary cortical neurons, STHdh$^{Q7/7}$ cells and immortalised astrocytes). To determine whether αsyn caused cell death via ferroptosis, αsyn was co-administered with ferroptosis inducers (iron and buthionine sulphoxamine), and ferroptosis inhibitors (desferrioxamine, reduced-glutathione, ferrostatin-1 and liproxstatin-1). The impact of these ferroptosis modulators on the toxicity of αsyn was compared to their impact on the toxicity of erastin (potent ferroptosis inducer). Ferroptotic markers (lipid peroxidation, iron and glutathione levels) were measured in the intoxicated cells.
Results: Liproxstatin-1 (potent ferroptosis inhibitor) dose-dependently abolished αsyn and erastin toxicity. However, unlike erastin, αsyn toxicity was not modulated by any of the other inducers or inhibitors of ferroptosis. This implied that αsyn causes cell death by a ferroptosis-independent pathway, despite the complete rescue by liproxstatin-1. We hypothesise that liproxstatin-1 binds αsyn to confer neuroprotection; and indeed, αsyn aggregation was inhibited by liproxstatin-1.
Conclusion: That αsyn and iron act independently to cause death in cell models of PD. Liproxstatin-1 could be a compound that protects against both types of lesion.

Poster 141 – Tuesday 6th December

HYPOTHALAMIC AND PONTINE NEUROMODULATORY SYSTEMS: FOCUS ON NUCLEUS INCERTUS INTERACTIONS IN THE RAT

Azadeh Sebatghadam[1,2,3], Caitlin E Singleton[1,4], Alan Kania[5], Aga Grabowiecka[5], Marcin Siwiec[5], Emma KE Ong-Pålsson[1,2], Anna Blasiak[5], Sherie Ma[1,2], **Andrew L Gundlach[1,2,4]**

[1]The Florey Institute of Neuroscience and Mental Health, [2]Florey Department of Neuroscience and Mental Health, [3]Department of Medicine, Austin Health, [4]Department of Anatomy and Neuroscience, [5]Department of Neurophysiology and Chronobiology, Jagiellonian University

Integrated function of neuromodulatory circuits is required for optimal physiological and behavioural responses to the internal/external environment. Neural signalling networks involved include brainstem monoamine systems and multiple hypothalamic neuropeptide systems. Similarly, GABA/peptide neurons in the *nucleus incertus* (NI), including those containing relaxin-3, innervate and influence regions/circuits involved in arousal/vigilance [1], metabolism [2], and reward/motivation [3,4] via interactions with CRF, orexin and oxytocin/(vasopressin) signalling [2-5], but their precise interactions with hypothalamic circuits have not been elucidated.
Therefore, in adult male rats, we examined: (*i*) the neurochemistry of NI neurons, identifying distinct GABA/relaxin-3 and GABA/CCK populations; (*ii*) the comparative innervation pattern of NI neurons by orexin and MCH neurons, identifying inputs that varied in intensity with the light/dark cycle; (*iii*) the respective excitatory and inhibitory responses to orexin and MCH (600 nM) of NI neurons *in vitro*; (*iv*) the effect of intra-NI administration of orexin-A or MCH on locomotor activity and feeding, with increased and decreased locomotor activity (2h) and food-intake (4h), respectively, after intra-NI orexin-A and MCH (600 pmol; 5-10/group, $P \leq 0.05$); and (*v*) the innervation of hypothalamus by NI/relaxin-3 neurons, with sparse contacts observed between NI/relaxin-3 inputs and MCH/orexin neurons in lateral hypothalamus. These studies further highlight the functional diversity of NI neurons.

1. Ma S et al. (2016) *Brain Struct Funct* doi:10.1007/s00429-016-1230-0.
2. Ganella DE et al. (2013) *Gene Ther* 20:703-716.
3. Kastman HE et al. (2016) *Neuropharmacology* 110:82-91.
4. Walker LC et al. (2016) *Addiction Biol* doi:10.1111/adb.12426.
5. Ma S et al. (2013) *J Physiol (Lond)* 591:3981-4001.

Poster 142 – Monday 5th December

NOCICEPTOR NEURONS ON MICROFLUIDIC-BASED CHIPS – A FIRST STEP TOWARDS A MULTIPURPOSE PAIN SENSOR

Dr Dusan Matusica[1], Dr Tommy Tong[2], Jastrow Canlas[1], Professor Nicolas Voelcker[2], **Prof Rainer Viktor Haberberger[1]**

[1]Anatomy & Histology, Centre for Neuroscience, Flinders University, [2]ARC Centre of Excellence for Convergent Bio-Nano Science and Technology, Future Industries Institute, University of South Australia

Purpose: Pain and chronic pain after injury or nerve damage are universal health problems for society, about as costly as diabetes and cancer combined. Treatments are often ineffective, partly because neither the reason for pain, nor the response to treatment can be adequately determined. Therefore new diagnostic tools are urgently needed to measure pain and help to identify new therapeutic targets for better, more effective treatment.
Methods & Results: We established a robust protocol to differentiate the rat embryonic dorsal root ganglion cell line 50B11 into peptidergic nociceptive neurons. We validated the significant changes into a peptidergic phenotype by morphology (length and number of processes), detection of mRNAs (qRT-PCR, TRPV1, P2X3, TrkA, n = 5) and presence (Western Blot, TRPV1, CGRP, P2X3, TrkA n = 5) and location (immunohistochemistry, CGRP, SP, P2X3, n = 5) of proteins characteristic for peptidergic nociceptors. In addition, 50B11 cells could be cultured and differentiated into nociceptors on microfluidic chips (n = 3). Using multi-channel microfluidics, nociceptors were simultaneously activated with agonists for peptidergic and non-peptidergic nociceptors and morphological changes monitored over time, followed by staining an immunohistochemical analysis. Conclusion: Using a virtually pure population of peptidergic nociceptive neuron-like cells we were able to create a microfluidic bio-nano-chip interface which will enable us to monitor and evaluate simultaneously the action of several agents such as drugs or patient serum on pain sensing neurons and therefore pain signaling.

Poster 143 – Tuesday 6th December

CIRCULATING OXIDATIVE STRESS INDICATORS CHANGE FOLLOWING TRAUMATIC BRAIN INJURY IN A PILOT STUDY

Alison Halstrom[1], Ellen MacDonald[2,3], Claire Neil[3], Glen Arendts[2,3], Daniel Fatovich[2,3], Melinda Fitzgerald[1]

[1]University Of Western Australia, [2]Royal Perth Hospital, [3]Centre for Clinical Research in Emergency Medicine

Traumatic brain injury (TBI) encompasses a broad range of injury mechanisms, severity, and outcomes, and is often acquired in combination with extra-cerebral trauma. All of these factors make determination of TBI severity a complex challenge, with tools currently available to clinicians insufficient to tailor a clinical response to individual patients' needs. Blood biomarkers of TBI may supplement existing clinical assessments, but currently available biomarkers have limited sensitivity or specificity. While oxidative stress is known to feature in damage mechanisms following TBI, investigation of blood biomarkers of oxidative stress following these injuries has been limited. A small exploratory study of a subset of trauma patients with TBI of varying severity has been conducted. Circulating concentrations of the astro-glial damage indicators S100b, and myelin basic protein (MBP) were quantified, as well as biomarkers of oxidative stress including; hydroxynonenal (HNE), malondialdehyde (MDA), carboxymethyl-lysine (CML), and 8- hydroxy-2'-deoxy-guanosine (8-OHDG). Significant increases in circulating S100b, MBP and HNE, and a decrease in CML, were observed in TBI patient samples (n = 18) compared to uninjured age and gender matched controls (n = 8) ($p \leq 0.05$), with all of these but MBP displaying trends towards severity dependent variation. This small exploratory study supports the current literature on S100b and MBP elevation in TBI, and reveals potential for the use of peripheral oxidative stress markers to assist in rapid, quantitative determination of TBI severity. Further investigation is required to validate results and confirm trends.

Poster 144 – Monday 5th December

SYNAPSE DYSFUNCTION OF LAYER V PYRAMIDAL NEURONS PRECEDES NEURODEGENERATION IN A MOUSE MODEL OF TDP-43 PROTEINOPATHIES

Miss Emily Handley[1], Dr Kimberley Pitman[1], Dr Edgar Dawkins[1], Dr Kaylene Young[1], Miss Rosemary Clark[1], Miss Tongcui Jiang[1], Dr Bradley Turner[2], Associate Professor Tracey Dickson[1], Dr Catherine Blizzard[1]

[1]Menzies Institute For Medical Research, [2]The Florey Institute of Neuroscience and Mental Health

TDP-43 is the major component of pathological neuronal inclusions in frontotemporal dementia and amyotrophic lateral sclerosis, with mutations associated with disease onset and progression. Whilst research has previously focused on the nuclear role of TDP-43 and aggregates, recent evidence indicates mis-processing pathologically impacts the synapse. We report an important role for TDP-43 in cortical dendritic spine formation at th compartment. Immunohistochemistry, western blots, electrophysiology and spine density analysis were utilised at post-natal (P) days 30, 60 and 90 in Thy1-YFPH and Thy1-YFPH::TDP-43^{A315T} mice. Dendritic spine density and morphology was investigated in 20µm coronal sections using confocal microscopy and Neurolucida software. Dendritic spine density in the motor and somatosensory cortices of Thy1-YFPH mice increased from P30, peaking at P60, prior to pruning at P90. C, density was reduced in the motor cortex of Thy1-YFPH::TDP-43^{A315T} mice prior to symptom onset and cell loss at P60, and in the motor and somatosensory cortices at symptom onset (P90) a cell loss. Morphological spine-type analysis revealed impairment in the development of mushrooms spine within the motor cortex of Thy1-YFPH::TDP-43^{A315T} mice. Furthermore, dendritic spine alterations corresponded to lowered efficacy of synaptic transmission at P60 determined by electrophysiology. ogether, the findings suggest mutated TDP-43 has a significant pathological effect at the dendritic spine, associated with decreased efficacy of neural transmission and plasticity. Cranial window surgical techniques and 2photon liveimaging will now be utilised to further investigate this early disease event in TDP-43 proteinopathies.

Poster 145 – Tuesday 6th December

INVESTIGATING THE FREQUENCY-DEPENDENT EFFECTS OF LOW-INTENSITY REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (LI-RTMS) ON HUMAN MOTOR CORTICAL EXCITABILITY

Ms Katherine Hankinson[1], Associate Professor Jennifer Rodger[2], Emeritus Professor Geoff Hammond[3], Ms Kalina Makowiecki[2]

[1]School of Human Biology, Anatomy and Physiology UWA, [2]School of Animal Biology UWA, [3]School of Psychology UWA

Low-Intensity rTMS (LI-rTMS) is a non-invasive form of brain stimulation that induces structural and functional changes in disordered cortical circuits in mice (Rodger et al. 2012) and reduces depressive symptoms in humans with treatment-resistant depression (Martiny, Lunde & Bech 2010). LI-rTMS may also contribute to the effects of other forms of non-invasive brain stimulation by changing cortical excitability. The purpose of this study was to investigate the frequency-dependent effects of LI-rTMS on human motor cortical excitability, comparing four stimulation conditions (10Hz, BHFS, cTBS and sham) after a single stimulation session. We also aimed to determine whether different factors may modulate LI-rTMS induced effects, and how these factors may contribute to variability of results.

We measured cortical excitability by recording motor evoked potentials (MEPs) from the hand muscle before and after LI-rTMS. LI-rTMS did not significantly change cortical excitability and no frequency-dependent effects of LI-rTMS were found after a single stimulation. However, an interesting time of day effect was demonstrated, with a significant increase in cortical excitability variability occurring in the morning compared to the afternoon for all stimulation conditions.

This study demonstrated that LI-rTMS does not significantly alter motor cortical excitability or exhibit frequency-dependent effects in humans. Further research is required to investigate whether LI-rTMS can influence the brain when using different stimulation parameters. An important finding from the current study was the influence of time of day on cortical excitability. This has implications for future research and clinical application of non-invasive brain stimulation.

Poster 146 – Monday 5th December

EXCITOTOXIC MECHANISMS IN AXON DEGENERATION

Miss Kelsey Hanson[1], Dr Carmen Fernandez-Martos[1], Dr Nan Tian[1], Professor James Vickers[1], Dr Anna King[1]

[1]Wicking Dementia Research And Education Centre, University of Tasmania

Objective: Axon degeneration is a potential therapeutic target for Alzheimer's disease, however, mechanisms of axon degeneration in neurodegenerative diseases are not well characterized. One potential driving mechanism of axon degeneration is excitotoxicity, which can cause breakdown of the microtubule cytoskeleton, although mechanisms by which this occurs are currently unknown. Therefore, we examined modification of microtubules and associated proteins after kainic acid induced excitotoxicity.
Methods: Primary cortical culture of embryonic day 15.5 C57Bl/6 mice were grown to 10 days in vitro (DIV) prior to treatment with high (100µM) and low (25µM) concentrations of kainic acid to induce excitotoxicity. Using ELISA and immunocytochemical techniques, tubulin post-translational modification levels were measured at 1, 6 and 18 hours post-treatment. In particular, modifications known to stabilize and destabilize microtubules such as acetylation and tyrosination were measured to determine if they were affected after excitotoxic insult.
Results: Treatment with kainic acid induced beading and fragmentation of axons, which was dose dependent. There was a significant activation of the apoptotic protein caspase 3 within the axon even at low levels of kainic acid. Preliminary analysis indicated that there was no significant difference ($p>0.05$, $n=3$) in the tyrosination of tubulin, which is associated with destabilizing, at 1, 6, and 18 hours following treatment with high and low levels of kainic acid treatment. Future studies will investigate whether other microtubule modifications occur following excitotoxicity.
Conclusion: These data suggest that microtubule breakdown following excitotoxicity is not the result of altered tyrosination to microtubules.

Poster 147 – Tuesday 6th December

S100B IS IMPORTANT FOR MAINTAINING NEURAL PRECURSORS DURING DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

Dr Marlene Hao[1], Ms Elena Capoccia[1,2], Dr Carla Cirillo[1], Dr Werend Boesmans[1], Prof Pieter Vanden Berghe[1]

[1]University Of Leuven, [2]Sapienza University

Introduction: S100B is expressed in various types of glial cells and appears to be involved in regulating many aspects of their function. However, little is known about its role during nervous system development. In this study, we investigated the effect of inhibiting the onset of S100B synthesis in the development of the enteric nervous system (ENS), a network of neurons and glia located in the wall of the gut that is vital for control of gastrointestinal function. **Methods:** Gut explants were taken from embryonic day (E)13.5 mice, the day before the first immunohistochemical detection of S100B, and cultured in the presence of arundic acid (300 µM), an inhibitor of S100B synthesis, for 48 hours. **Results:** After 48 hours, many S100B-immunoreactive cells could be detected in vehicle control cultures. Arundic acid successfully inhibited S100B synthesis as no, or very few, S100B+ cells could be detected in its presence. Culture in arundic acid also resulted in a reduction in the proportion of Sox10+ enteric neural crest progenitors and a decrease in ENS progenitor proliferation. There was no change in the density of HuC/D+ enteric neurons, however, a small population of cells (approx. 10%) exhibited atypical co-expression of Sox10 and HuC/D, which was not observed in control cultures. Addition of exogenous S100B to the cultures failed to rescue the change in Sox10+ cell numbers. **Conclusion:** S100B appears to be important for maintaining Sox10 expression and consequently, promoting progenitor proliferation, during ENS development. The longer-term effects of this depletion require further investigation.

Poster 148 – Monday 5th December

MEASURING EFFECTIVE ELECTRODE AREA AND CHARGE DENSITY OF PLATINUM ELECTRODES FOR COCHLEAR IMPLANTS

Dr Alex Harris[1,4], Paul Carter[2,4], Dr Carrie Newbold[3,4], Prof Robert Cowan[3,4], Prof Gordon Wallace[1,4]

[1]Intelligent Polymer Research Institute, University Of Wollongong, [2]Cochlear Ltd, [3]Department of Otolaryngology, University of Melbourne, [4]The HEARing CRC

Cochlear implants provide auditory perception to individuals with profound hearing loss. Implant recipients have poor amplitude perception due to limited dynamic range, and minimal discrete frequency perception. The current implant is composed of 22 platinum electrodes. New electrode materials and designs have been proposed to increase dynamic range and frequency perception with little clinical benefit to date. This indicates improvement of the implant requires a greater understanding of the electrode-tissue interface, in particular the charge transfer process at the platinum surface. Electrochemical methods have been used to measure the effective electrode area and charge density. The effective electrode area was measured by hydride adsorption or by reduction of a redox species using cyclic

voltammetry. Typically a hydride adsorption density of 210 □C/cm^2 from a Pt(100) crystal plane is used to calculate the electrode area. However, the platinum electrode is polycrystalline, with hydride adsorption giving a broad reduction current. Hydride adsorption density on polycrystalline platinum is variable and may be significantly different to a Pt(100) single crystal. Calculation of electrode area assuming a hydride adsorption density of 210 □C/cm^2 allows comparison of electrodes of the same design but does not provide accurate effective electrode area values. Electrode areas measured by reduction of $Ru(NH_3)_6^{3+}$ is insensitive to crystal structure and provides accurate effective area values. Current methods for measuring effective electrode area and charge density of cochlear implants are inaccurate. Measurement of electrode area by reduction of $Ru(NH_3)_6^{3+}$ provides more accurate values and may allow improvement of electrode materials and designs.

Poster 149 – Tuesday 6th December

NFIX IS REQUIRED FOR MULTIPLE STAGES OF ADULT-BORNE HIPPOCAMPAL NEURON DIFFERENTIATION

Mr Lachlan Harris[1], **Dr Oressia Zalucki[1,2]**, **Professor Richard Gronostajski[3]**, **Associate Professor Michael Piper[1,2]**

[1]School Of Biomedical Sciences, The University of Queensland, [2]Queensland Brain Institute, The University of Queensland, [3]Department of Biochemistry, State University of New York

[#]These authors contributed equally to this work

The *Nfi* family of transcription factors (NFIA, NFIB, NFIC and NFIX) are critical for the timely differentiation of neurons and glia during the development of the cortex, cerebellum and spinal cord. It has recently been postulated through enhancer annotation of BMP4 treated neural stem cells (NSCs) *in vitro*, that NFIX also functions as one of the central transcription factors mediating NSC quiescence, a feature unique to adult NSCs that prevents excessive proliferation and ensures the long term survival of this population. Here, through *in vivo* genetic analysis, we find that while loss of NFIX results in a modest increase in NSC proliferation, this has no effect on the long-term maintenance of NSCs. Rather, there is an absolute requirement for NFIX expression in transitioning intermediate hippocampal progenitors to become neuroblasts (early neuroblast differentiation), and later, the branching of neuroblast dendritic processes (late neuroblast differentiation). As a result, mice lacking NFIX fail to form mature adult-borne hippocampal neurons and subsequently display a deficit in learning and memory. These results are the first to investigate the function of NFIX, or any of the NFI proteins during adult hippocampal neurogenesis, and demonstrate the absolute requirement for NFIX in the differentiation of adult-borne neurons.

Poster 150 – Monday 5th December

CELL TYPE-SPECIFIC EXPRESSION OF NFIX IN THE DEVELOPING AND ADULT CEREBELLUM

Mr James Fraser[1], Ms Alexandra Essebier[2], Mr Kevin Mutemi[1], Professor Richard Gronostajski[5], Dr Mikael Boden[2], Professor Brandon Wainwright[3], **Dr Tracey Harvey[1]**, Associate Professor Michael Piper[1,4]

[1]The School of Biomedical Sciences, University of Queensland, [2]The School of Chemistry and Molecular Biosciences, University of Queensland, [3]The Institute for Molecular Bioscience, University of Queensland, [4]Queensland Brain Institute, [5]Department of Biochemistry, Program in Genetics, Genomics and Bioinformatics, Center of Excellence in Bioinformatics and Life Sciences, State University of New York at Buffalo

Transcription factors from the Nuclear Factor One (NFI) family have been shown to play a central role in regulating neural progenitor cell differentiation within the embryonic and postnatal brain. NFIA and NFIB, for instance, promote the differentiation and functional maturation of granule neurons within the cerebellum. Mice lacking Nfix in all cells exhibit delays in the development of neuronal and glial lineages within the cerebellum, but the cell type-specific expression of this transcription factor remains undefined. We examined the expression of NFIX, together with various cell type-specific markers, within the developing and adult cerebellum using co-immunofluorescence labelling and confocal microscopy. Embryonically, progenitor cells within the rhombic lip and ventricular zone expressed NFIX. Postnatally, progenitor cells within the external granule layer, as well as migrating and mature granule neurons, expressed NFIX. Within the adult cerebellum, NFIX displayed a broad expression profile, and was evident within granule cells, Bergmann glia and interneurons, but not within Purkinje neurons. Furthermore, transcriptomic profiling of cerebellar granule neuron progenitor cells showed that multiple splice variants of Nfix are expressed within this germinal zone of the postnatal brain. Collectively, these data suggest that NFIX plays a role in regulating progenitor cell biology within the embryonic and postnatal cerebellum, as well as an ongoing role within multiple neuronal and glial populations within the adult cerebellum.

Poster 151 – Tuesday 6th December

HYPERPHOSPHORYLATED TAU REDUCES ACTION POTENTIAL FIRING BY RELOCATING THE AXON INITIAL SEGMENT

Dr Robert Hatch[1], Dr Yan Wei[1,2], Mr Di Xia[1], Professor Jürgen Götz[1]

[1]Queensland Brain Institute, [2]Institute of Biophysics Chinese Academy of Sciences

Hyperphosphorylated tau characterizes many neurodegenerative disorders and has been shown to impair neuronal function. How hyperphosphorylated tau reduces synaptic activity has been investigated intensively; however, its effect on neuronal excitability has received less attention. Here we show using patch clamp electrophysiology that hyperphosphorylated tau induces a more depolarized

threshold for action potential initiation and reduces firing in hippocampal CA1 neurons from two complementary tau transgenic mouse models. Furthermore, using mutagenesis and primary hippocampal neuronal cultures, we demonstrate that this reduction in neuronal excitability results from a relocation of the axon initial segment in a tau phosphorylation- and microtubule-dependent manner. Pharmacological stabilization prevents both the structural and functional deficits caused by tau hyperphosphorylation. These data provide insights into the pathogenesis of neurodegenerative diseases involving the tau protein and into the plasticity of the axon initial segment.

Poster 152 – Monday 5th December

CHARACTERISATION OF HUMAN APOE25 FRAGMENT IN A CELL CULTURE MODEL

Dr Li Henry[1], Ms Kalani Ruberu[1], Ms Sonia Sanz Muñoz[1], Dr Lezanne Oo1[1], Dr Brett Garner[1]

[1]Illawarra Health and Medical Research Institute and School of Biological Science, Faculty of Science, Medicine and Health, University of Wollongong

It is established that the apoE4 isoform is associated with increased Alzheimer's disease (AD) risk. We previously identified a stable ~25 kDa apoE fragment (apoE25) in human post-mortem brain samples and we discovered that apoE25 levels were two- to three-fold higher in apoE3 compared to apoE4 subjects (1); thereby implying a protective role for apoE25. Analysis of apoE25 function and homeostasis has been hampered by the lack of a suitable cell culture model; indeed apoE25 has not been previously detected *in vitro*. Here we assessed possible apoE25 production by SK-N-SH neuroblastoma cells treated (3 to 50 days) with all-trans retinoic acid (ATRA, 10 □M), a protocol that induces neuronal differentiation. In agreement with earlier studies (2), full-length apoE3 was expressed in untreated SK-N-SH cells. Addition of ATRA induced apoE25 generation from day 3, reaching a peak at day 9, with levels maintained for as long as 50 days. ApoE25 production was correlated with increased levels of beta-III tubulin and neurofilament markers of differentiation. ApoE25 was not detected in the cell lysates, whereas an extracellular protease inhibitor significantly inhibited apoE25 production, suggesting apoE25 is an extracellular proteolytic fragment of secreted full-length apoE. ApoE25 was further purified by heparin affinity chromatography and structural analysis confirmed the presence of a sialylated glycan, confirming characteristic apoE N-terminal features. Purification of cell culture-derived apoE25 will now permit amino acid sequence analysis and functional studies to be conducted.

1. Elliott DA *et al.* (2011) *Neurobiol Aging* 32:257-271.
2. Elliott DA *et al.* (2010) *BMC Neurosci* 11:23.

Poster 153 – Tuesday 6th December

SMALL BRAINS DEALING WITH A DIVERSITY OF EYES AND VISUAL OPSINS - THE DYNAMIC EVOLUTIONARY HISTORY OF VISION IN PANCRUSTACEANS.

Dr. MJ Henze[1], Prof. TH Oakley[2]

[1]Queensland Brain Institute, University Of Queensland, [2]Department of Ecology, Evolution and Marine Biology, University of California

Compared to vertebrates, pancrustaceans (Crustacea and Hexapoda) possess only small brains to deal with the sensory information provided by cephalic eyes. At the same time, they display an enormous diversity of eye designs, including multiple types of compound eyes and single-chambered eyes, often with colour vision and/or polarization vision. We have examined the evolutionary history of eyes and visual opsins across the principle groups of Pancrustacea. First, we reviewed the distribution of lateral and median eyes, which are found in all major pancrustacean groups, but are lacking in some taxa of each of those groups. We then compiled data on the expression of visual r-opsins (rhabdomeric opsins) in lateral and median eyes across Pancrustacea and found no evidence for ancient opsin clades expressed in only one type of eye. Instead, opsin clades with eye-specific expression are products of recent gene duplications, indicating a dynamic past, during which opsins often changed expression from one type of eye to another. Finally, we investigated the evolutionary history of r-opsins. From analysing a reconciled, phylogenetic tree of arthropod r-opsins, we infer that the ancestral pancrustacean had at least four visual opsin genes. These are the progenitors of opsin clades that later were variously duplicated or lost during pancrustacean evolution. Together, our results reveal a particularly dynamic history, with losses of eyes, numerous duplications and losses of opsin genes, and changes in opsin expression between types of eyes. Ultimately, this flexibility on the retinal level might reflect a peripheral matched-filter solution advantageous for small brains.

Poster 154 – Monday 5th December

CHARACTERIZATION OF TWO TYPES OF RHYTHMIC ELECTRICAL BEHAVIOUR IN MOUSE COLONIC SMOOTH MUSCLE

Tim Hibberd[1], Prof. Marcello Costa[1], Lee Travis[1], Prof. Simon Brookes[1], Prof. Nick Spencer[1]

[1]Flinders University & Centre for Neuroscience

In this study we characterized electrical activity of the muscularis externa in mouse colon. Flat sheet preparations of whole mouse colon were set up in organ baths superfused with oxygenated Krebs solution, serosa uppermost. Muscle electrical activity was recorded with extracellular suction electrodes. Ongoing discharge of smooth muscle firing was recorded in all preparations. Firing was organized into discrete, rhythmic bursts. Two types of events were observed: neurogenic, as they were abolished by hexamethonium or TTX, and

myogenic, as they persisted in these drugs. Neurogenic events comprised large bursts of firing, averaging 31±11s duration, at 2.1±0.5Hz (n=6). Myogenic events comprised smaller bursts of action potentials (10±1s) at 0.9±0.1Hz (n=6). Ongoing neurogenic events occurred at intervals of 349±256s (n=6), while myogenic events occurred every 35±6s (n=6). Neurogenic, but not myogenic events were promptly evoked by mechanical probe applied to the rectum (15mN von Frey hair, 17/20 tested, n=3). Myogenic events were abolished by removal of the longitudinal muscle, or by nicardipine, but persisted in hexamethonium and/or TTX. In comparison, ongoing neurogenic events were abolished by hexamethonium or TTX but persisted after removing longitudinal muscle. The effects of mechanical probing on neurogenic events were abolished in hexamethonium (0/8 tested, n=3). Electrical oscillations lacking action potentials but with similar frequency and intervals to neurogenic events were readily detected in nicardipine. Thus, we distinguish two patterns of muscle activation. Neurogenic events may underlie colonic propulsive contractions, while myogenic events may correspond to myogenic "ripples" previously identified in guinea pig proximal colon.

Poster 155 – Tuesday 6th December

INSULA-CINGULATE INTERPLAY CORRELATES TO HIGH-EFFORT BEHAVIOUR IN RAT

Dr Kristin Hillman[1], Professor David Bilkey[1]

[1]University Of Otago

In human imaging studies, neural activity increases in the anterior cingulate cortex (ACC) and the anterior insula (AI) as tasks become more effortful. It is not well-understood, however, just how increases in ACC and AI activity affect effort expenditure. Here we recorded ACC-AI local field potentials in rats as they ran a continuous T-maze that presented differential effort demands on each side of the T-junction. We found that as effort demands increased, power spectral densities (PSDs) in the 2-30 Hz range increased correspondingly in both the ACC and AI. In the central stem of the T-maze, where animals were likely to be processing the upcoming choice options, we discovered that a relative increase in ACC power over AI power predicted a high-effort choice option, whereas the opposite relationship predicted upcoming effort avoidance. This finding suggests that dynamic power differentials between the ACC and AI correlate to choice behaviour when it comes to driving, or dissuading, effort expenditure in a task.

Poster 156 – Monday 5th December

ALTERED NEUROIMMUNE FUNCTION IN A MOUSE MODEL OF AUTISM: INCREASED SMALL INTESTINAL TRANSIT AND REDUCED CAECAL WEIGHT IN NEUROLIGIN-3 R451C MICE

Ms Anita Leembruggen[1], Ms Fátima Ramalhosa[2], Ms Gracia Seger[1], Associate Professor Ashley Franks[3], Professor Joel Bornstein[1], **Dr Elisa Hill-Yardin[1]**

[1]Department of Physiology, The University Of Melbourne, [2]Life and Health Sciences Research Institute (ICVS), Minho University, Campus de Gualtar, [3]Department of Microbiology, La Trobe University

Gastrointestinal dysfunction and alterations in inflammatory markers are common in Autism spectrum disorder (ASD) patients however the underlying cause is unknown. Many synaptic gene mutations, including in Neuroligin genes are implicated in ASD and patients with the R451C missense mutation in Neuroligin-3 (NL3) have chronic diarrhoea and oesophagitis. In order to determine if small intestinal function is altered due to the R451C mutation, 8 week old $NL3^{R451C}$ mice were fed Carmine red dye by gavage and the distance to the dye front along the small intestine measured after 10 min. Small intestinal length, stomach and caecal weight were recorded. Caecal weight (and microbial content) is an indicator of immune function. In NL3 mutant mice, the ratio of the dye front distance: total small intestinal length was higher compared to WT (0.46 ± 0.03 and 0.58 ± 0.04 cm, n = 17 and 18, WT and NL3 respectively; p=0.01). No changes in small intestinal length between genotypes (40.8 ± 3.1 and 39.1 ± 3.9, WT and NL3 respectively, p = 0.11) or stomach weight (0.40 ± 0.04 and 0.44 ± 0.04 g, WT and NL3 respectively, p=0.41) were observed. Interestingly, caecal weight was markedly reduced in NL3 mutants versus WT mice (0.70 ± 0.04 and 0.50 ± 0.02; p<0.0001). These novel findings suggest that the NL3 R451C mutation affects enteric neuronal communication and potentially plays a role in inflammatory processes in these mice.

Poster 157 – Tuesday 6th December

SPONTANEOUS SYMPATHETIC BAROREFLEX SENSITIVITY IS REPEATABLE IN HEALTHY YOUNG INDIVIDUALS AT REST.

Miss Sarah L Hissen[1], Miss Khadigeh El Sayed[2], Professor Vaughan G Macefield[2,3], Doctor Rachael Brown[2], Doctor Chloe E Taylor[1,2]

[1]School of Science and Health, Western Sydney University, [2]School of Medicine, Western Sydney University, [3]Neuroscience Research Australia

Spontaneous sympathetic baroreflex sensitivity (BRS) is a valuable tool used to assess how well the baroreflex buffers beat-to-beat changes in blood pressure. Sympathetic BRS is quantified through the relationship between diastolic blood pressure and muscle sympathetic nerve activity (MSNA). To date, there is little research on the repeatability of this approach. In 54 healthy young individuals, continuous measurements of blood pressure, heart rate and MSNA were recorded for two 5-min minute periods within the same session. Sympathetic BRS was quantified using both MSNA burst incidence (BRS_{inc}) and total MSNA (BRS_{total}). Significant sympathetic BRS_{inc} slopes were obtained for both 5-min periods in 49 participants. There was no significant difference in sympathetic BRS between the first (-3.1 ± 0.2% bursts/mmHg) and the second (-3.1 ± 0.3% bursts/mmHg; P= 0.64) 5-min period. Significant sympathetic BRS_{total} slopes were obtained for

both 5-min periods in 37 participants. There was also no difference in sympathetic BRS_{total} between the first (-4 ± 0.4AU/beat/mmHg) and second (-3.9 ± 0.4 AU/beat/mmHg; P= 0.88) 5min period. Bland-Altman plots revealed that there was small negative bias for BRS_{inc} (-0.03% bursts/mmHg) and BRS_{total} (-0.12 AU/beat/mmHg) suggesting good agreement between the two BRS slopes. Results from this study indicate that measures of spontaneous sympathetic BRS are repeatable within the same session in young healthy adults.

Poster 158 – Monday 5th December

DECIPHERING THE ROLE OF PROTEIN PHOSPHORYLATION IN CEREBRAL TYPE I INTERFERONOPATHIES

Barney Viengkhou[1,2], Melanie Y White[1,3], Iain L Campbell[1,2], Stuart J Cordwell[1,3], **Markus J Hofer[1,2]**

[1]School of Life and Environmental Sciences and the Charles Perkins Centre, The University of Sydney, [2]Bosch Institute, The University of Sydney, [3]School of Medical Sciences, The University of Sydney

Interferon-α (IFN-α) is a member of the type I IFN family of cytokines. It is central to regulating host immune responses. Dysregulated production of IFN-α in the central nervous system (CNS) is the cause of several neurological diseases, collectively termed "cerebral type I interferonopathies". They include chronic viral infections, systemic lupus erythematosus-associated encephalopathy and the genetic disorder Aicardi-Goutières syndrome. The molecular mechanisms underlying these diseases are largely unknown. Importantly, the effects of IFN-α are mediated through a number of signalling pathways that are regulated by phosphorylation of signalling proteins and transcription factors. We aimed to identify the protein phosphorylation cascades (the 'phosphoproteome') responsible for the neurological diseases caused by IFN-α using high-throughput phosphoproteomics. To determine the global IFN-α phosphoproteome in the CNS, transgenic mice with CNS-targeted production of IFN-α (GIFN mice) were used. GIFN mice recapitulate many of the key features of human cerebral type I interferonopathies. In addition, IFN-α-treated primary astrocytes and microglia were used to assess cell-type specific changes within the phosphoproteome.
Our results show that there was a significant change in the phosphorylation of more than 800 proteins in the CNS of GIFN mice. These proteins were associated with a diverse range of cellular processes, suggesting that the IFN-α-induced phenotype is mediated through several different pathways. Surprisingly, analysis of phosphorylation sites revealed that members of the mitogen-activated protein kinase (MAPK) family are major regulators of protein phosphorylation in GIFN mice. Our results help to clarify how IFN-α causes neurological disease.

Poster 159 – Tuesday 6th December

BRAIN REGION SPECIFIC ALTERATIONS IN THE TRANSCRIPTION FACTOR CREB IN FOLATE AND MTHFR DEFICIENT MICE

Mr Alexander Hoffman[1], Dr. Jean-Marie Sontag[1], Dr. Brandi Wasek[2], Dr. Teodoro Bottiglieri[2], Dr. Estelle Sontag[2]

[1]School of Biomedical Sciences and Pharmacy, Faculty of Health and Hunter Medical Research Institute, The University of Newcastle, [2]Institute of Metabolic Disease, Baylor Research Institute

Common polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR)* gene and dietary folate deficiency can lead to alterations in folate metabolism. Significantly, the brain is particularly sensitive to folate metabolic disturbances, and low plasma folate status is a risk factor for many neurological disorders. Population-based studies have demonstrated that a low plasma folate status is associated with cognitive decline. Impairment of learning and memory is also recapitulated in mouse models of severe MTHFR deficiency. Notably, signal-dependent activation of cAMP-responsive element binding protein (CREB)-dependent gene transcription is known to play a key role in learning, memory and plasticity. Here, we investigated CREB status in brain regions from young (5-week-old) and old (22-month-old) mouse models of mild and/or or severe MTHFR deficiency, and mice fed a control or low folate diet (6 mice per group). In these models, we found significant alterations in the protein expression ($p < 0.01$) and/or phosphorylation ($p < 0.001$) levels of CREB in cerebellar, midbrain, hippocampal and cortical brain regions. These results demonstrate an important and complex link between genetic and dietary alterations in folate metabolism and signalling pathways that regulate learning and memory

Poster 160 – Monday 5th December

MUTANT CCNF-BASED ZEBRAFISH MODEL OF MOTOR NEURON DISEASE

Miss Alison Hogan[1], Dr Emily Don[1], Dr Angela Laird[1], Miss Stephanie Raynor[1], Miss Maxinne Watchon[2], Miss Kristy Yuan[1], Miss Serene Gwee[1], Dr Albert Lee[1], Miss Claire Winnock[1], Miss Jennifer Fifita[1], Dr Kelly Williams[1], Dr Garth Nicholson[1], Dr Roger Chung[1], Dr Ian Blair[1], Dr Nicholas Cole[1]

[1]1Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Macquarie University, [2]2Department of Anatomy and Histology, Sydney Medical School, University of Sydney

is a rapidly progressing, fatal neurodegenerative disease. Approximately 10% of cases have a known family history of the disease and mutations in multiple genes have been identified,providing insight into pathogenesis. Our laboratory recently identified -linked mutations in *CCNF*, however the pathological mechanisms these mutations are yet to be established.
Zebrafish ideal model investigat mechanisms and potential therapeutics *in vivo.* Transient overexpression of mutant *CCNF* mRNA in zebrafish embryos produced models with an impaired motor response to a light stimulus (photomotor response). A significant motor

axonopathy was identified in these embryos and a significant correlation demonstrated between reduced axonal length and impaired motor function, suggesting a causal relationship. This finding was supported by an *in vitro* study, which demonstrated reduced neurite outgrowth in Neuro-2a cells overexpressing mutant *CCNF.* Further analysis of the mutant zebrafish models also revealed elevated levels of apoptosis in the spinal cord. This was also supported by an *in vitro* study. Proteomic analysis of the *CCNF*-transfected Neuro-2a cells revealed significant disruption in cell stress and survival pathways.
The zebrafish model presented in this study is the first animal model based on a *CCNF* mutation. Characterisation of the model indicates that it will be a useful tool to examine the pathogenesis of mutant *CCNF*-induced motor neuron degeneration, particularly the neurite outgrowth and apoptosis pathways. This model suit preliminary testing of potential therapeutics aimed at modifying these pathways.

Poster 161 – Tuesday 6th December

INVESTIGATING THE ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN ALCOHOL ADDICTION: STUDIES IN BDNF HETEROZYGOUS MUTANT RATS

Mr Samuel Hogarth[1], Professor Maarten van den Buuse[1], Doctor Emily Jaehne[1], Doctor Elvan Djouma[1]
[1]*La Trobe University*

Alcoholism is a chronic disease characterized by compulsive alcohol abuse, causing 3.3 million deaths worldwide annually. In Australia, 18% of people will suffer an alcohol-related injury during their lifetime. Brain derived neurotrophic factor (BDNF) is a neurotrophin involved in neuronal cell survival, brain development, and synaptogenesis. BDNF has also been implicated in aspects of addiction. However, the role of BDNF in alcoholism remains unclear.

To test this relationship, male and female BDNF heterozygous mutant rats and matched wildtype (WT) Sprague-Dawley controls (n=4-8 per group) were trained to self-administer alcohol (10% v/v) using operant chambers (Med Associates). The animals undertook daily 20 min sessions to assess their preference to alcohol (fixed ratio schedule, 3 presses=1 reward), their determination to obtain an alcoholic reward (progressive ratio), and their resilience to reward extinction and cue-induced reinstatement.

Both female and male rats pressed the active lever significantly more often than the non-active lever, with approximately 70-80% preference for alcohol over the water control ($p<0.001$). There were no differences between BDNF heterozygous rats and WT controls in alcohol preference. Progressive ratio analysis similarly revealed no significant genotype difference in breakpoint and extinction and cue-induced reinstatement were similar in the two genotypes of either sex.
In conclusion, despite having only half the normal levels of BDNF expression, BDNF heterozygous mutant rats showed no difference with WT controls in any of the stages of this operant paradigm. Further studies are underway to assess the relationship between activity-dependent BDNF release and alcoholism in BDNF val66met polymorphic animals.

Poster 162 – Monday 5th December

VELOCITY DETECTION ABILITIES OF THE BLOWFLY IN NATURAL AND ARTIFICIAL IMAGES

Miss Marissa Holden[1]

[1]*Flinders University*

Neural computation of velocity in natural scenes is a challenging, yet essential task for Dipteran flies to successfully navigate through their environment aerially. Natural images contain more complex characteristics compared to artificial images and vary vastly in spatial structure and contrast. Research has identified a high degree of predictability in natural scenes however, making natural and artificial images an interesting comparison when investigating motion processing. The horizontal system (HS) neuron in the lobula plate of the blowfly, *Calliphora*, gives directionally-selective responses to horizontal motion and has been previously identified to code for velocity. We here aim to determine whether *Calliphora* has an increased ability to detect deviations in velocity in natural images compared to artificial images by recording intracellularly from the HS neuron using a sharp electrode. During intracellular recordings, *Calliphora* were presented with four panoramic images (two natural and two artificial), which moved horizontally in the neuron's preferred direction at two different base velocities that corresponded to different aerial behaviours of the fly. Deviations from the base velocity were presented for 0.5 seconds and then images continued to move at the original base velocity for 1.5 seconds. Using this method, we have found, that the HS neuron shows a significant depolarization in response to velocity deviations (increases in addition to decreases) of 8 degrees per second in both natural and artificial images, whereas at deviations of 3 degrees per second there was a substantial dependence on base velocity (N=3).

Poster 163 – Tuesday 6th December

EFFECT OF MATERNAL SEPARATION ON ETHANOL CONSUMPTION: INVESTIGATING THE ROLE OF ALPHA4 CONTAINING NICOTINIC RECEPTORS

Miss Joan Holgate[1], Professor Selena Bartlett[1]

[1]***Queensland University Of Technology***

Exposure to multiple traumatic early life events dramatically increases the risk of developing alcohol use disorders (AUDs). Alpha4 containing (*) nicotinic acetylcholine receptors (nAChRs) modulate alcohol seeking behaviours and have been identified as a target for treating AUDs. They are also altered by stress and capable of modulating corticosterone levels. However, little is known about the mechanisms involved. To examine the effect of early life stress on ethanol consumption and the brain regions alpha4* nAChRs may be important, YFP-tagged alpha4* nAChR mice underwent maternal (MS) or maternal and sibling (MPS) separation for 3hrs daily (P2-14). From 6 to 18 weeks of age the mice consumed 20% ethanol or water using the 2-bottle choice drinking in the dark paradigm. Brain samples were collected for western blotting analysis of alpha4* nAChR expression. Exposure to ethanol did not affect body weigh in either sex. Experiencing MS and MPS produced a significant reduction ($p<0.0001$) in body weight compared to controls in male offspring only, which persisted with increasing age. In female offspring, MS and MPS did not alter ethanol consumption however, in males MS and MPS increased ethanol consumption (*30mins:* $p<0.0001$, controls 1.58±0.04 g/kg/30min, MS 1.95±0.03 g/kg/30min, MPS 1.75±0.05 g/kg/30min; *2hrs:* $p<0.0001$, controls 4.58±0.08 g/kg/2hr, MS 5.50±0.07 g/kg/2hr, MPS 4.97±0.09 g/kg/2hr). Alpha4* nAChR expression varied with brain region, sex and exposure to ethanol and early life stress. Our findings indicate that alpha4* nAChRs are involved in the stress response and pharmacological manipulation of these receptors could be useful for preventing stress driven alcohol consumption.

Poster 164 – Monday 5th December

A PULVINAR CONNECTION TO SCHIZOPHRENIA

Doctor Jihane Homman-ludiye[1], Mr Martin Burgess[2], Professor Cindy Shannon Wieckert[2], Associate Professor James Bourne[1]

[1]Australian Regenerative Medicine Institute, [2]Neuroscience Research Australia

The pulvinar is the largest thalamic nucleus in primates, including humans, comprising multiple subregions. Research to date has primarily focussed on its role in visual processing and relaying of sensory information between cortical areas. The medial portion of the pulvinar (PM), however, has reciprocal connectivity with the dorsolateral prefrontal cortex (DLPFC), an area of the neocortex known to be impaired in patients with schizophrenia. To date, little attention has been given to the potential role of the pulvinar in schizophrenia, although human studies have demonstrated a reduction in volume and specific neuronal loss in PM. Unfortunately, these studies utilised adult tissue and therefore, provide limited knowledge on the abnormal developmental events leading to schizophrenia. In the developing marmoset monkey, we proposed to characterise the ontogeny of PM at the cellular, molecular and connectivity levels. First, we mapped its connectivity in the adult with MRI-guided tracer injections (n=2), revealing distinct topography between anterior and posterior regions of PM. Next, we investigated the origins of the neurones populating PM (n=2), identifying the neurogenic sites they migrate from, genes expressed and the timing of their migration. This comprehensive profile of PM development will then be compared with molecular and cytoarchitectural analyses performed in parallel in the PM of schizophrenic patients, providing a new understanding of the role of PM in schizophrenia but also, the cellular and molecular mechanisms underpinning these defects.

Poster 165 – Tuesday 6th December

EXPLORING THE SIGNALLING MECHANISM OF GLUTAMATE EXCITOTOXICITY IN CULTURED PRIMARY NEURONS BY QUANTITATIVE PROTEOMIC AND PHOSPHOPROTEOMIC APPROACHES

Ashfaqul Hoque[1], M Iqbal Hossain[1], Ching-Seng Ang[2], Nicholas A Williamson[2], Dominic CH Ng[3], Heung-Chin Cheng[1]

[1]Biochemistry and Molecular Biology Department, University of Melbourne, [2]Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, [3]Faculty of Medicine and Biomedical Sciences, University of Queensland

Excitotoxicity, the overstimulation of ionotropic glutamate receptors is a key process of neuronal loss in acute ischaemic stroke and chronic neurological disorders. However, exactly how neuros dye in excitotoxicity still remains unclear. Using stable-isotope dimethyl labelling based quantitative proteomic and phosphoproteomic approaches, we identified at least 80 neuronal proteins showing perturbed expression and 59 phosphoproteins showing significant changes in phosphorylation following 15 min and 4 h of glutamate-induced excitotoxicity. Most of the identified neuronal proteins exhibited reduced expression in excitotoxicity. Signalling network analysis using IPA with these identified protein molecules revealed (i) 14-3-3-mediated signalling, (ii) remodelling of epithelial adherens junctions, (iii) cell cycle including G2/M DNA damage checkpoint regulation, (iv) Myc-mediated apoptosis signalling, (v) PI3K/Akt signalling and (vi) Erk/MAPK signalling as top dysregulated canonical pathways in excitotoxicity. Using similar approach, we also identified significantly dysregulated neuronal proteins and phosphoproteins that are downstream of neurotoxic GluN2B-containing extrasynaptic NMDA receptors. Representative proteomic data were validated by Western blot analysis, also changes in phosphorylation of Mef2c (Ser-222), Mff (Ser-146), Mlf2 (Ser-237) and Stmn1 (Ser-38) were validated by label-free full-scan precursor ions (MS1) quantitation analysis using isotopically labelled synthetic phosphopeptide standards. Our results collectively indicate that inactivation of a number of pro-survival signalling pathways and activation of a series of pro-death signalling pathways cooperate to cause neuronal demise in excitotoxicity. In summary, our findings shed light on the molecular mechanism of excitotoxic neuronal death and identified neuronal proteins are potential targets for the development of neuroprotectants to reduce excitotoxic brain damage in neurological disorders

Poster 166 – Monday 5th December

CORTICAL PROCESSING OF BINOCULAR INFORMATION IN THE MOUSE

Dr Helena Huang[1], Professor Greg Stuart[1]

[1]*Eccles Institute Of Neuroscience, JCSMR, ANU*

Visual information arising from the two eyes is integrated in the primary visual cortex (V1). Given that the vast majority of retinal projections (>95%) crosses the mid-line, we surmised that cortical input from the ipsilateral eye is mediated via an indirect callosal projection from the contralateral V1. Using urethane anaesthetised adult C57BL6/J mice, we performed whole-cell recordings from pyramidal cells in the binocular region of V1 while illuminating either or both eyes using light-emitting diode (LED) "goggles". As expected, neuronal responses were biased towards the contralateral eye (ocular dominance index = 0.56±0.07; n=30 cells). Importantly, we found that excitatory postsynaptic potentials evoked by illuminating the ipsilateral eye were delayed relative to those evoked by illuminating the contralateral eye (onset latency difference = 16.6±2.3 ms; n=19 cells), consistent with the notion that the ipsilateral input arises via a distinct synaptic pathway from the contralateral input. In a different set of experiments, tetrodotoxin (TTX) was injected into V1 on one side while recording the local field potentials from V1 in both hemispheres. TTX injections abolished responses from either eye in the injected hemisphere and significantly reduced the ipsilateral eye response in the opposite hemisphere (39±14% reduction, n=3 mice). Taken together, our results suggest that the ipsilateral visual input to binocular V1 contains a significant callosal projection from the contralateral V1. Experiments are currently underway to quantify the contribution of this callosal component to binocular processing of visual information using optogenetics in conjunction with whole-cell recordings.

Poster 167 – Tuesday 6th December

FEEDBACK FROM HIGHER-ORDER VISUAL CORTICAL AREA AFFECTS SPIKE-RESPONSES OF NEURONS IN CAT'S 'INTERMEDIATE' CORTEX

Dr Jin Y Huang[1,2], Dr Chun Wang[2], Professor Bogdan Dreher[2]

[1]*Discipline of Biomedical Science, The University of Sydney,* [2]*Discipline of Anatomy and Histology, The University of Sydney*

Postero-temporal visual (PTV) cortex is a higher-order pattern/form-processing area of the cat. A subset of its neurons sends direct 'feedback' projection to the ipsilateral cytoarchitectonic area 19 (V3). In order to study the role of these recurrent projections on spike-activities of area 19 neurons, PTV cortex was reversibly inactivated. **Methods**: Four animals were anesthetised and pharmacologically immobilised. Extracellular background spike-activities and spike-responses to visual stimuli of single area 19 neurons were recorded before cooling (PTV-36˚C), during cooling (PTV-10˚C) and after rewarming (PTV-36˚C) of ipsilateral PTV. **Results**: Transient inactivation of PTV very rarely resulted in significant reductions in background spike-activity of V3 cells. Substantial (≥ 20˚) shifts in preferred orientation and/or substantial (≥ 20˚) changes in width of orientation tuning curves were uncommon. By contrast, in a substantial proportion of V3 neurons, cooling of PTV resulted in: i) significant reversible changes (reduction or increases) in peak magnitude of spike-responses to visual stimuli (35.5%; 10/28); ii) substantial reversible changes in their direction selectivity indices (43%; 12/28) and iii) reversible, upward shifts in their preferred stimulus velocities (37%; 7/19). **Conclusion**: Higher-order pattern/form-processing visual cortical signals tend to play an important role in determining the magnitude of spike-responses and some 'motion-related' receptive field properties of a proportion of neurons of an 'intermediate' form-processing visual area 19.

Poster 168 – Monday 5th December

THE PROTEIN EXPRESSION OF PACAP AND ITS RECEPTOR (PAC1) IN THE HUMAN INFANT BRAINSTEM AND HIPPOCAMPUS

Jessica Huang[1], Professor Karen Waters[1], Dr Rita Machaalani[1]

[1]*Faculty of Medicine, The University Of Sydney*

Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide that plays an important role in cardiorespiratory regulation. One way of doing this is through the activation of its receptor PAC1. To date, the expression of PACAP and PAC1 in the human infant brain has not been studied. The aim of this study is to report on their expression within the human infant brainstem medulla, pons, midbrain and hippocampus. This was via immunohistochemistry on formalin-fixed paraffin embedded tissue from n=12 infant cases. For PACAP, we found high expression in the motor nuclei of the medulla hypoglossal (XII), dorsal motor nucleus of vagus (DMNV), inferior olive nuclues (ION) and arcuate nucleus (AN), moderate in the nuclei of the pons and midbrain, and the lowest in the nucleus of the solitary tract (NTS) and inferior colliculus (IC). High expression in the cornus ammonius 4, 3 and 2 region of the hippocampus and the lowest expression was noted in the subiculum (SUB) and dentate gyrus (DG). For PAC1, we found high expression in the AN, with lowest expression in the NTS and DMNV. In the hippocampus, low expression was found throughout all CA regions. Therefore, this study is the first to determine the expression of PACAP and PAC1 in the human brainstem and hippocampus showing that PACAP is high in motor neurons important in respiratory control thus supporting data that PACAP plays a functional role in maintaining respiration.

Poster 169 – Tuesday 6th December

THE UNFOLDING PROTEIN RESPONSE IN OREXIN/DYNORPHIN NEURONS IN SUDDEN INFANT DEATH SYNDROME (SIDS): P-PERK-MEDIATED LOSS OF TRANSLATION ASSOCIATED WITH NEURODEGENERATIVE PATHWAYS

Mr Nicholas Hunt, Prof Karen Waters, Dr Rita Machaalani
[1]*Department of Medicine, Sydney Medical School,* [2]*BOSCH Institute of Biomedical Research,* [3]*The Children's Hospital*

Sudden Infant Death Syndrome (SIDS) infants have decreased orexin immunoreactivity within the hypothalamus and pons. This study examined multiple neurodegenerative mechanisms that promote loss of orexin expression. Immunofluorescence and immunohistochemical staining for a number of markers was performed in the tuberal hypothalamus and pons of infants (1-10 months) who died from SIDS (n = 27) compared to age and sex matched non-SIDS infants (n = 19). The markers included: orexin A (OxA), dynorphin (Dyn), cleaved caspase 3 (CC3), cleaved caspase 9 (CC9), glial fibrillary acid protein (GFAP), tubulin beta chain 3 (TUBB3), myelin basic protein (MBP), interleukin 1β (IL-1β), terminal-deoxynucleotidyl-transferase-dUTP-nick-end-labelling (TUNEL), c-fos and the unfolding protein response (UPR) activation markers: phosphorylated protein kinase RNA-like endoplasmic reticulum kinase (pPERK) and activating transcription factor 4 (ATF4). Within the hypothalamus, OxA and Dyn co-localized with a 20% decrease in expression in SIDS infants ($P = 0.001$). pPERK and ATF4 in OxA neurons were increased by 35% ($P = 0.001$) and 15% ($P = 0.001$) respectively, with linear relationships evident between the decreased OxA/Dyn expression and the percentages of co-localised pPERK/OxA and ATF4/OxA ($P = 0.01$, $P = 0.01$). No differences in co-localisation with CC9, CC3, TUNEL or c-fos, nor expression of MBP, TUBB3, IL-1β, and GFAP were observed in the hypothalamus. In the pons, there were 50% and 20% increases in pPERK expression in the locus coeruleus ($P = 0.001$) and dorsal raphe ($P = 0.022$) respectively. ATF4 expression was not changed. Thus, loss of orexin in SIDS infants was associated with an accumulation of pPERK, indicating loss of translation. The results also suggest promotion of an UPR stress pathway. Finally, UPR may inhibit multiple neuronal groups (serotonin, noradrenaline) in SIDS leading to a common pathway that promotes loss of protein expression and impaired functionality of these neuronal groups.

Poster 170 – Monday 5th December

ROLE OF THE VOLTAGE-GATED POTASSIUM CHANNEL SUBUNIT KV8.2 IN INHERITED RETINAL DISEASE AND INTERACTION WITH OTHER CHANNEL PROTEINS.

Associate Professor Nathan Hart[1], Dr Jessica Mountford[2,3], Assistant Professor Livia Carvalho[2], Associate Professor Wayne Davies[2,3], **Professor David Hunt[2,3]**

[1]Department of Biological Sciences, Macquarie University, [2]Lions Eye Institute, University of Western Australia, [3]School of Animal Biology, University of Western Australia

A unique form of cone-rod dysfunction arises from mutations in the *KCNV2* gene which encodes the voltage-gated potassium channel subunit Kv8.2. The ERG for this disorder shows a severe reduction in the amplitude of a- and b-waves at lower light intensities which changes to an enhanced b-wave at higher intensities. This unusual ERG is diagnostic for the disorder. Kv8.2 only forms functional channels with another channel protein, Kv2.1, encoded by *KCNB1*. We now have mouse knock-outs for both these genes. The Kv8.2 KO mimics the human disorder in showing a severely depressed ERG which then flips to an enhanced b-wave response at higher light intensities. At all light intensities, the implicit time for the b-wave is prolonged. The Kv2.1 KO and double KO show similar patterns, although in neither case does the b-wave rise above normal. The a-wave in all three genotypes remains severely depressed, and the c-wave is totally absent. Photopic flicker is also reduced and significantly delayed. Our results show that the Kv8.2 KO is an excellent model of the human disorder. In addition, we have shown that loss of Kv2.1 results in a similar phenotype. The implications of these observations on the function and interaction of these Kv subunits in the regulation of phototransduction will be discussed, together with potential treatment regimens.

Poster 171 – Tuesday 6th December

CELLULAR ORIGINS OF ILLNESS-INDUCED ANHEDONIA: AN EXAMINATION OF INTER-STRAIN LPS-INDUCED NUCLEUS ACCUMBENS CFOS EXPRESSION

Ms Krystal Iacopetta[1], Mrs Lyndsey Collins-Praino[1], Mrs Femke Buisman-Pijlman[1], Professor Mark Hutchinson[1,2]

[1]University Of Adelaide, [2]Centre for Nanoscale Biophotonics

Anhedonia, a key feature of many psychiatric disorders, is an acute or chronic deficit in the capacity to feel pleasure. Development of anhedonia has been attributed to dysregulation of reward pathways yet clear understanding of the neurobiological mechanisms responsible for the balance of hedonia/anhedonia is lacking. Experimentally, anhedonia can be using two-bottle choice (2BC) preference testsroject aimedcharacterise response. Strain specific anhedonic behaviour was (cFos activation) within the nucleus accumbens. Temporal recording of licking at maximal preference robustly demonstrated inter-strain variability in the preference for saccharin. When challenged with endotoxin (LPS) a significant reduction in the preference was observed in the C57/BL6 and CBA strains ($p<0.05$) but no Balb/C mice. Preliminary tissue analysis of the nucleus accumbens showed LPS increased cFos activation compared to saline controls but this was not comparative to the behavioural variation of anhedonia displayed between strains ($n = 3$ per group).

Poster 172 – Monday 5th December

SENSORY EXPERIENCE MODIFIES FEATURE MAP RELATIONSHIPS IN VISUAL CORTEX

Dr Shaun Cloherty[1,2], Mr Nicholas Hughes[3], Dr Markus Hietanen[1,2], Dr Partha Bhagavatula[1,2], Professor Geoffrey Goodhill[3], **Professor Michael Ibbotson[1,2]**

[1]National Vision Research Institute, [2]ARC Centre of Excellence for Integrative Brain Function, Department of Optomtery and Vision Sciences, University of Melbourne, [3]Queensland Brain Institute, The University of Queensland

The extent to which brain structure is influenced by sensory input during development is a critical but controversial question. A paradigmatic system for studying this is the mammalian visual cortex. Maps of orientation preference (OP) and ocular dominance (OD) in the primary visual cortex of ferrets, cats and monkeys can be individually changed by altered visual input. However, the spatial relationship between OP and OD maps has appeared immutable. Using a computational model we predicted that biasing the visual input to orthogonal orientation in the two eyes should cause a shift of OP pinwheels towards the border of OD columns. We then confirmed this prediction by rearing cats wearing orthogonally oriented cylindrical lenses over each eye. Thus, the spatial relationship between OP and OD maps can be modified by visual experience, revealing a previously unknown degree of brain plasticity in response to sensory input.

Poster 173 – Tuesday 6th December

INTERACTION BETWEEN NOVEL OSCILLATION WITHIN THE VENTROMEDIAL HYPOTHALAMUS AND THE SYMPATHETIC NERVOUS SYSTEM

MD And PhD Kamon Iigaya[1,2], MD and PhD Yoshino Minoura[3], PhD Hiroshi Onimaru[1]

[1]Department of Physiology, Showa University School of Medicine, [2]Department of Internal Medicine, Hiratsuka city Hospital, [3]Departments of Medicine, Division of Cardiology, Showa University School of Medicine

The ventromedial hypothalamus (VMH) is known to play an important role in feeding behavior and sympathetic nerve activity (SNA) control. We report the identification of novel neuron groups that showed around 0.06 Hz oscillations on both sides of the VMH on hypothalamus slice preparations from rats using electrophysiological and optical imaging. Glucose sensing neurons in the VMH were confirmed by change of glucose concentration. The frequency of oscillation was increased decrease by superfusion with a low high glucose concentration. The VMH neurons express receptors of various neuropeptides that are involved in the regulation of feeding behavior. The application of some feeding peptides induced frequency changes in VMH oscillation, similar to the changes in the SNA induced by the application of those compounds in brain. The VMH oscillation frequency also corresponded to a low range of heart rate variability which is one of the indexes of SNA. The VMH activity, phrenic nerve and thoracic sympathetic nerve trunk activity were simultaneously recorded in the decerebrated and arterially perfused *in situ* rat preparation. Power spectral analysis in the *in situ* perfused preparation revealed similar peak values within the lowfrequency between the VMH oscillation and sympathetic nerve trunk activity. We hypothesize that the VMH oscillation is involved in SNA and that its regulation could be a new strategy for control of energy expenditure and eventually obesity.

Poster 174 – Monday 5th December

ALTERED BEHAVIOURAL AND COGNITIVE FUNCTION IN P38DELTA-DEFICIENT MICE

Dr Arne Ittner[1], Josefine Bertz[1], Prof Lars Ittner[1]

[1]School of Medical Sciences, UNSW Australia

p38 MAP kinases are involved in many processes that are essential to mammalian physiology. Of the 4 mammalian p38 genes p38alpha, p38beta, p38gamma and p38delta, only p38alpha has been studied in behavioural responses and cognition. Here, we studied cognitive, behavioural and motor performance in p38delta knockout mice using motor (accelerating Rotarod, pole test), spatial memory (Morris-water-maze, differential paired associate learning), recognition memory (visual discrimination task), anxiety/inhibition (elevated plus maze, openfield paradigm), locomotion (openfield paradigm) and attention (5-choice serial reaction time task) tests. Strikingly, p38delta knockout mice show markedly reduced anxiety, reduced performance in spatial memory tasks, increased locomotion/activity and reduced attention, suggesting a role of p38delta that affects multiple behavioural and cognitive outcomes. Surprisingly, we could not detect p38delta expression in different brain regions nor in neurons. High levels of p38delta, however, were found in the adrenal cortex, suggesting a peripheral (adrenocortical) function of p38delta that impacts on behaviour and cognition. Thus, p38delta has a non-redundant function among p38 MAP kinases in regulation of behaviour and cognition.

Poster 175 – Tuesday 6th December

ARX PA2 MICE SEIZE THE DAY: CHARACTERISING THE PHENOTYPE OF EXPANDED POLYALANINE TRACT MUTATIONS.

Dr Matilda Jackson[1,2], Dr Kristie Lee[1,2], Tessa Mattiske[1,2], Dr Emily Jaehne[3], Prof Bernhard Baune[3], A.Prof Nigel Jones[4], A.Prof Cheryl Shoubridge[1,2]

[1]Intellectual Disability Research, School of Medicine, The University Of Adelaide, [2]Robinson Research Institute, The University of Adelaide, [3]School of Medicine, Discipline of Psychiatry, The University of Adelaide, [4]Department of Medicine, University of Melbourne

The *Aristaless*-related homeobox gene (*ARX*) is a known intellectual disability gene that frequently presents with X-linked Infantile Spasm Syndrome as a comorbidity. ID with epilepsy in children is a chronic and devastating disorder that has poor treatment options and disease outcomes. To gain a better understanding of the role that *ARX* plays in ID and epilepsy, we investigate *ARX* patient mutations modelled in mice. Over half of all *ARX* mutations result from expansions of the first two polyalanine (PA) tracts. However, phenotypic data for the mouse modelling the more frequent PA2 mutation in patients (c.428_451dup) has not been reported and constitutes a barrier to understanding

the molecular mechanisms involved. Here we report the first comprehensive analysis of postnatal outcomes for both PA1 and PA2 mouse models. Both strains were found to have impaired learning and memory, reduced activity, increased anxiety and reduced sociability; with PA1 mice generally displaying greater behavioural deficits in keeping with the more severe phenotype reported in patients. In agreement with Kitamura *et al.* (2009), 70% of PA1 males exhibit myoclonic seizures by two months of age, with the first observed at P18. In this report we show 80% of PA2 males also display myoclonic seizures, with the first observed at P19. Consistent with patient phenotypes, we observe large variations in seizure progression and severity for both PA1 and PA2 individual mice. The generation of this comprehensive baseline data is a necessary step on the path to the development of intervention therapies to improve patient outcomes.

1. Kitamura et al., Hum Mol Genet 2009 18(19):3708-24

Poster 176 – Monday 5th December

MICROANATOMY OF FEAR EXTINCTION WITHIN SUBREGIONS OF THE PREFRONTAL CORTEX AND AMYGDALA REVEALED BY ARC AND pERK/MAPK ACTIVITY

Ms Angela Jacques[1,2], Mr Nicholas Chaaya[1,2], Mrs Marie Carmody[1,2], Ms Chiemi Hettiarachchi[2], Dr Andrew Battle[2,3], Dr Luke Johnson[1,2,4]

[1]Queensland University of Technology, School of Psychology and Counselling, [2]Queensland University of Technology, Institute of Health and Biomedical Innovation, Translational Research Institute, [3] Queensland University of Technology, School of Biomedical Science, [4]Uniform Services University School of Medicine, Center for the Study of Traumatic Stress, Department of Psychiatry

Post-traumatic stress disorder (PTSD) is a memory disorder of enhanced fear responding and reduced fear extinction. Traumatic events involve key aspects of Pavlovian association between aversive and neutral stimuli (Johnson et al, 2012 Psychopharm). In order to understand and successfully treat PTSD a complete understanding of the micro-anatomy and molecular events underlying extinction is needed. Following associative memory (CS+US x3), we performed an 'extended extinction'; rats received 3 days (d) of 20xCS, then 3d home cage for consolidation prior to test. Both phospho-ERK/MAPK (pMAPK) and Arc protein (Arg 3.1/Arc) are sequentially activated in response to memory. We combined Arc and pMAPK immunoreactivity to measure micro-circuit localization of memory. On memory test day (3xCS) extinction animals showed reduced responding to the CS (freezing 10.17 +/- 0.73%, n=10) compared to controls (47.17 +/- 7.25%, n=10) (p=0.0352), suggesting animals were fully extinguished. The relative contribution of pMAPK and ARC were measured in LH and RH hemispheres respectively (n = 6 animals). We found 54% pMAPK vs 46% Arc within the LA and 52% vs 48% within the IL. These results indicate numerically similar contributions of pMAPK and ARC activity in neurons occur during extinction memory recall within the LA (p=0.8133) and IL (p=0.8584). Future studies need to investigate whether the same or parallel neural microcircuits simultaneously express pMAPK and ARC activity during extinction memory recall. These data reveal patterns of activity with in microcircuits of the LA and IL cortex and begin to identify the spatial and temporal properties of extinction memory micro-circuits.

Poster 177 – Tuesday 6th December

A ROLE OF SEROTONIN IN METHAMPHETAMINE PSYCHOSIS? STUDYING THE EFFECT OF CHRONIC YOUNG-ADULT METHAMPHETAMINE ON BEHAVIOUR IN 5-HT1A RECEPTOR KNOCKOUT MICE

Dr Emily Jaehne[1], Ms Dzeneta Ameti[1], Ms Tehani Paiva[1], Ms Camilla Hume[1], Prof Maarten van den Buuse[1]

[1]La Trobe University

Objective: Methamphetamine is a widely abused stimulant drug but this abuse is associated with an increased risk of developing psychosis. In addition to its well-known action on brain dopamine, methamphetamine also affects serotonergic (5-HT) neurons. Here we address the possible role of brain serotonin in methamphetamine psychosis by studying its effects in mice which lack the 5-HT1A receptor.

Methods: Male and female wildtype (WT) or 5-HT1A knockout mice (1AKO) received daily treatment with increasing doses of methamphetamine from 6-9 weeks of age. At least two weeks after the last injection, the mice underwent a battery of behavioural tests focusing on psychosis-related behaviours.

Results: As expected, an acute methamphetamine challenge increased locomotor activity to a greater extent in methamphetamine-pretreated mice compared to controls (P<0.001), reflecting sensitization. Methamphetamine-pretreated mice also spent more time on the open arms of the elevated plus maze (P=0.007), suggesting reduced anxiety. These results were not dependent on 5HT1A genotype. In the social interaction test most mice showed preference for interacting with a stranger mouse, however this sociability tended to be reduced by prior methamphetamine treatment in female WT, but not 1AKO mice. Such an effect was not observed in males. There were no changes in social recognition memory or prepulse inhibition (PPI) in any of the groups.

Conclusions: The results show a number of effects of chronic young-adult methamphetamine on behaviour in adulthood. With the possible exception of sociability in female mice, these effects did not reveal an involvement of serotonin, at least not the 5-HT1A receptor.

Poster 178 – Monday 5th December

THE ROLE OF VITAMIN D IN COGNITION AND BRAIN FUNCTION IN ADULT C57BL/6J AND APP23 MICE

Kara Jaeschke[1], Professor Juergen Goetz[2], Associate Professor Thomas Burne[1,3]

[1]Queensland Brain Institute, The University of Queensland, [2]Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, [3]Queensland Centre for Mental Health Research, The Park Centre for Mental Health

Recent evidence has indicated that low vitamin D levels during adulthood may be related to cognitive impairment and neurodegenerative disorders such as Alzheimer's disease (AD). The primary aim of this research was to investigate the effect of vitamin D on cognition and brain function in healthy mice and a mouse model of AD.
Adult male C57BL/6J mice were exposed to varying levels of vitamin D (0, 150, 1,500 and 15,000 IU/vitamin D3/kg referred to as deficient, insufficient, control and elevated, respectively) for 10 weeks and then assessed on a hippocampal-dependent test for spatial learning and memory; active place avoidance (APA). Male and female APP23 mice - a transgenic mouse model of AD - exposed to control or elevated levels of vitamin D, were also assessed on APA. Both C57BL/6J and APP23 mice were tested for behaviours that could alter performance on the APA task. Proteomics was used to analyse protein expression in hippocampal tissue of APP23 mice.
Vitamin D status did not affect spatial learning and memory in C57BL/6J mice. Vitamin D also had no effect on behavioural domains in C57BL/6J and APP23 mice. However, supplementation with vitamin D impaired spatial performance in transgenic APP23 mice. The preliminary hippocampal proteomics analysis in APP23 mice suggested that elevated vitamin D was associated with differential protein expression, increased activation of upstream regulators and disruption of functional pathways. These results show that elevated vitamin D could potentially exacerbate the progression of AD and these findings warrant further scrutiny.

Poster 179 – Tuesday 6th December

ENDOCANNABINOIDS MEDIATE DEPOLARISATION-INDUCED SUPPRESSION OF EXCIATION IN CRH NEURONS

Mr Bradley Jamieson[1], Dr Karl Iremonger[1]

[1]Centre for Neuroendocrinology, Department of Physiology, University of Otago

Corticotropin-releasing hormone (CRH) neurons control the mammalian stress response. The activity of these neurons is regulated by upstream excitatory glutamatergic inputs. In other neural populations, synaptic depolarisations increase the target neurons' intracellular calcium concentration and induce the retrograde release of endocannabinoids. Endocannabinoids typically act through the type 1 cannabinoid receptor (CB1R) to inhibit neurotransmitter release from the upstream neuron. While endocannabinoid-mediated depolarisation-induced suppression of excitation (DSE) has been shown in other neurons, it is yet to be demonstrated in identified CRH neurons. To determine whether CRH neurons exhibit DSE, we performed whole-cell patch-clamp recordings from identified CRH neurons in brain slices from CRH-cre/tdTomato mice. We find that following a 5 second depolarisation to +20mV, the amplitude of evoked excitatory postsynaptic currents (eEPSCs) is significantly reduced (23.9 ± 7.1% inhibition, $p < 0.01$, $n = 20$). Bath application of the CB1R antagonist AM251 (5 μM) attenuated this inhibition to 10.5 ± 6.3% ($p = 0.8$, $n = 10$). Further, we mimicked the DSE effect with bath application of the CB1R agonist WIN55,212-2 (WIN, 2 μM). WIN significantly inhibited eEPSCs by 32.7 ± 9.6% ($p < 0.05$, $n = 10$). Together, these data show that CRH neurons exhibit DSE and that it is mediated by the CB1R. This suggests that CRH neurons can control their own excitation through endocannabinoid messengers. This microcircuit offers an interesting target when exploring stress-related disorders where CRH neurons are hyperactive

Poster 180 – Monday 5th December

L-THEANINE SPARES STRIATAL NEURONS FROM 3-NP INDUCED STRIATAL NEUROTOXICITY: ROLE OF STRIATAL NEUROTRANSMITTERS AND NITRIC OXIDE PATHWAY

Mr. Sumit Jamwal[1], Dr. Puneet Kumar[1]
[1]ISF College of Pharmacy, Moga

Aim and Background: L-theanine is unique amino acid which readily crosses blood brain barrier and posses neuroprotective potential against neurodegenerative disorders including Huntington Disease (HD). HD is characterized by selective loss of GABAergic medium spiny neurons. Therefore, the present study was intended to investigate the effect of L-theanine against 3-NP induced striatal toxicity.

Methods: Rats were administered with 3-NP for 21 days. L-theanine was given once a day, 1 hour prior to 3-NP treatment for 21 days and L-NAME (NOS inhibitor) and L-arginine (NOS activator) were administered 1 hour prior to L-theanine treatment. Body weight, behavioral parameters (locomotor activity, grip strength, narrow beam walk) observation was done on 1st, 7th, 14th and 21st day after 3-NP treatment. On 22nd day, animals were sacrificed and rat striatum was isolated for biochemical (mitochondrial complex- II, LPO, GSH and nitrite), pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and neurochemical analysis (GABA, Glutamate, DA, NE, 5-HT, DOPAC, HVA, 5-HIAA and Adenosine).

Results: 3-NP treatment significantly altered body weight, locomotor activity, motor coordination, mitochondrial complex-II activity, oxidative defence, pro-inflammatory mediators (TNF-α, IL-6 and IL-1β), and striatal neurotransmitters level i.e. GABA, glutamate, catecholamines, adenosine, inosine and hypoxanthine whereas L-theanine treatment (25 & 50 mg/kg/day, p.o.) significantly prevented these alterations. Concurrent treatment of L-NAME with L-theanine (25 mg/kg/day, p.o.) significantly enhanced protective effect of L-theanine (25 mg/kg/day, p.o.) whereas concurrent treatment of L-arginine with L-theanine (50 mg/kg/day, p.o.) significantly decreased the protective effect of L-theanine (50 mg/kg/day, p.o.).

Conclusion: The neuroprotective potential of L-theanine involves inhibition of detrimental nitric oxide production and prevention of neurotransmitters alteration in striatum.

Poster 181 – Tuesday 6th December

INVESTIGATION OF THE INNERVATION OF THE MURINE VAGINA IN RESPONSE TO INFLAMMATION

Miss Esther Ji[1], Miss Harman Sharma[1], Miss Pauline Yap[1], Mrs Pat Vilimas[1], Miss Melinda Kyloh[2], Professor Nicholas Spencer[2], Dr Christine Barry[1], Professor Rainer Haberberger[1]

[1]Anatomy & Histoloy, Centre for Neuroscience, Flinders University, [2]Human Physiology, Centre for Neuroscience, Flinders University

Persistent and recurrent pain of the vagina causes distress for a high proportion of women and available treatments are often ineffective. While increased innervation (hyperinnervation) of the vulvar region by presumptive nociceptors in vulvar vestibulitis syndrome (VVS) has been reported, the contribution of nociceptor dysfunction to VVS and related conditions of the lower urogenital tract is mostly unstudied. Therefore, the aim of this study was to investigate the impact of pro-inflammatory agent Complete Freund's Adjuvant (CFA) administration on the innervation of the murine vagina based on a new mouse model of vagina inflammation. Mice (n=4) injected with CFA into the vaginal wall were euthanized after 7 days, and multiple labelling immunohistochemistry was performed on 12µm sections from proximal and distal segments of the vagina. Sections (n=3 per animal) were analyzed using fluorescence microscopy and nerve fibres in the lamina propria identified by immunoreactivity for the pan-neuronal marker PGP9.5 and subsequently counted via image analysis and stereology. Compared with naïve mice (n=3), mice injected with CFA showed a significant increase in nerve fibres immunoreactive to PGP9.5 in lamina propria in both cervical and vulvar regions (ANOVA, $P<0.005$). With regards to the overall innervation in different regions of vagina, a characteristic decrease in density of nerve fibres in the vulvar region was observed compared with the cervical region of the vagina was observed in both groups. Future studies will analysis the neurochemical characteristics of these increased nerve fibres in mouse vagina in response to inflammation.

Poster 182 – Monday 5th December

ALTERATIONS TO NEURITE OUTGROWTH AND SYNAPSE DEVELOPMENT IN TDP-43A315T PRIMARY CORTICAL NEURONS

Miss Tongcui Jiang[1], Dr Edgar Dawkins[1], Miss Emily Handley[1], Assoc. Prof. Tracey Dickson[1], Dr Catherine Blizzard[1]

[1]Menzies Institute For Medical Research

Amyotrophic lateral sclerosis (ALS) is characterised by the progressive death of motor neurons in the central nervous system. Aggregated inclusions, predominately in neurons, characterise ALS pathologically and are frequently positive for the transactive response DNA-binding protein 43 (TDP-43). How TDP-43 confers neurotoxicity remains to be fully elucidated. Here we utilised the prpTDP-43^{A315T} : YFP mouse model expressing mutant TDP-43^{A315T} to investigate the effect of TDP-43^{A315T} on normal neuronal development and synapse formation. Primary cortical neurons, derived from individual E15.5 embryos were grown to 3, 5, 10 and 15 days in vitro (DIV). Whilst there was a significant ($p<0.05$) difference between wild type (WT) and TDP-43^{A315T} neurons in total and mean dendrite length at 15 DIV, dendrite branching number and order analysis indicated no difference in cell complexity between the two populations. However, dendrite spine quantification of WT and TDP-43^{A315T} neurons at 15 DIV demonstrated a significant ($p<0.05$) decrease in dendritic spine density in the TDP-43^{A315T} cells in comparison to WT controls. There was no significant ($p>0.05$) change in axonal outgrowth and growth cone areas, however the axonal growth cone dynamics was significantly altered by overexpression of TDP-43^{A315T}. This indicates that the TDP-43^{A315T} mutation may play a role in the formation of synaptic structures in cortical neurons. Unravelling the mechanisms that render neurons in the cortico-motor system vulnerable to TDP-43 pathology will be imperative in the pursuit of identifying novel therapeutic interventions.

Poster 183 – Tuesday 6th December

SUB-DIFFRACTIONAL TRACKING OF INTERNALIZED MOLECULES REVEALS HETEROGENEOUS DIFFUSIVE STATES OF SYNAPTIC VESICLES

Dr. Merja Joensuu[1], Dr. Pranesh Padmanabhan[1], Dr. Nela Durisic[1], Mr. Adekunle T. D. Bademosi[1], Dr. Liz Cooper-Williams[1], Dr. Isabel C. Morrow[1], Dr. Callista B. Harper[1], Mr. WooRam Jung[2], Prof. Robert G. Parton[2], Prof. Geoffrey J. Goodhill[3], Dr. Andreas Papadopulos[1], Prof. Frederic A. Meunier[1]

[1]Queensland Brain Institute, The University of Queensland, [2]Institute for Molecular Bioscience, The University of Queensland, [3]School of Mathematics and Physics, The University of Queensland

Our understanding of the endocytic pathway dynamics is severely restricted by the diffraction limit of light microscopy. To address this, we implemented a novel technique based on the sub-diffractional tracking of internalized molecules (sdTIM). This allowed us to image anti-GFP Atto647N-tagged nanobodies trapped in synaptic vesicles (SVs) from live hippocampal nerve terminals expressing VAMP2-pHluorin with 50 nm localization precision. Our results showed that, once internalized, VAMP2-pHluorin/Atto647N-nanobodies exhibited a markedly lower mobility than on the plasma membrane, an effect that was reversed upon restimulation in presynapses but not in neighboring axons. Using Bayesian model selection applied to hidden Markov modeling, we found that SVs oscillated between an immobile and two transport states of opposite direction. Importantly, once a SV entered the immobile state it was less likely to switch back to the transport states.

These results highlight the potential of the sdTIM technique to provide new dynamic insights into the dynamics of endocytic pathways in a wide variety of cellular settings.

Poster 184 – Monday 5th December

DELIVERY OF TRK C ANTIBODY TO MOTOR NEURONS OF NEONATAL MICE, VIA INTRAPERITONEAL INJECTION

Miss Lauren Jones[1], Miss Kalyani Huilgol[1], Professor Uri Saragovi[2], Dr Bradley Turner[3], Dr Mary-Louise Rogers[1]

[1]Department of Human Physiology, Centre for Neuroscience, Flinders University, [2]Department of Pharmacology and Therapeutics, McGill University, [3]The Florey Institute of Neuroscience and Mental Health, University of Melbourne

Background: We aim to establish the monoclonal antibody called 2B7[1] that targets the neurotrophin receptor Trk C, to determine its ability to target motor neurons from peripheral injections in the neonate. This is an important step to using 2B7 as part of our non-viral gene therapy[2] in the future.
Objectives: We hope to deliver labelled 2B7 to motor neurons of neonatal mice, via intraperitoneal (IP) injections. We also hope to determine dosing and length of time seen in motor neurons. **Methods**: 2B7 was labelled with ATTO-488[2]. PND1 C57BL/6-mice were injected IP with 2B7-488 and after euthanising (36h post-injection), the percent of spinal cord motor neurons labelled with 2B7-488, identified by ChAT. Presence of 2B7-488 was also determined, 48, 72, 168 hours after delivery (n=3 mice).
Results: 58.47+/-9.91% of motor neurons in neonatal mice (n=7) identified by ChAT was targeted by 2B7-488 36 h after IP injection of 150µg. 1-way ANOVA comparing 75µg, 150µg and 300µg (n=3-7 mice) showed significant difference to PBS injections (control) ($p<0.0001$). Half the dosage (75µg) targeted 27.80+/-7.53%, and double the dosage (300µg) targeted 43.74+/-7.10%. 2B7-488 (150µg) was maintained in 23.19+/-1.63% (n=3) of motor neurons until 168 hours (7 days).
Conclusion: Proof of concept for delivery of 2B7-488 to neonatal mice, with a maximum of 60% of motor neurons targeted with optimal dosage (150µg) was determined and 2B7 was maintained in motor neurons up to 7 days. The next step is to use this antibody in an immunogene[2].
References:
1: Brahimi, F., Maira, M., Barcelona, P. F., Galan, A., Aboulkassim, T., Teske, K., Rogers, M.-L., Bertram, L., Wang, J., Yousefi, M., Rush, R., Fabian, M., Cashman, N. & Saragovi, H. U. 2016. The Paradoxical Signals of Two TrkC Receptor Isoforms Supports a Rationale for Novel Therapeutic Strategies in ALS. *PloS one,* 11**,** e0162307.
2: Rogers, M.-L., Smith, K. S., Matusica, D., Fenech, M., Hoffman, L., Rush, R. A. & Voelcker, N. H. 2014. Non-viral gene therapy that targets motor neurons in vivo. *Frontiers in molecular neuroscience,* 7.
Affiliations:
1: Department of Human Physiology, Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, South Australia, 5001, Australia.
2: Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada
3: Department 3:The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville 3052, Victoria 3051, Australia

Poster 185 – Tuesday 6th December

INFILTRATION OF IMMUNE CELLS TO SITES OF SECONDARY NEURODEGENERATION AFTER ISCHEMIC STROKE

Kimberley Jones[1,2], Dr Maximilian Plank[1,2], Dr Steve Maltby[1,2], Laureate Professor Paul Foster[1,2], Professor Michael Nilsson[1,2,3], Associate Professor Frederick Rohan Walker[1,2,3]

[1]University Of Newcastle, [2]Hunter Medical Research Institute, [3]NHMRC Centre of Research Excellence Stroke Rehabilitation and Brain Recovery

We previously showed that experimental stroke led to microglia activation and neuronal loss in sites of secondary neurodegeneration distal to the infarct one month after injury. Studies have shown that immune cells are present in sites of infarction in the first hours and days after stroke and contribute to increased neurodegeneration in peri-infarct regions. However, it is not known if these cells are present in sites of secondary neurodegeneration, which emerge at later time points. This study aimed to investigate whether immune cells were present in the thalamus, the main site of secondary neurodegeneration, 14 days after photothrombotic stroke induction in mice. Thalamus were analysed using flow cytometry to determine levels of microglia, myeloid cells, CD4 T cells, CD8 T cells, B cells and neutrophils. We found significantly more microglia, CD4 T cells, CD8 T cells, B cells in the ipsilateral thalamus compared to the contralateral thalamus. Our results show that infiltrating immune cells persist in ischemic tissue after the acute ischemic phase is over, in sites of secondary neurodegeneration. As these cells have been shown to be detrimental to recovery after stroke, our findings have important implications for clinical research, as they suggest that inhibition of these cells may provide a promising therapeutic approach to resolving secondary neurodegeneration.

Poster 186 – Monday 5th December

THE ROLE OF THE ADENOSINE A2A RECEPTOR IN THE RODENT RETINA

Mr Gudmundur Jonsson[1], Dr Kirstan Vessey[1], Dr Thor Eysteinsson[2], Dr Erica Fletcher[1]

[1]Department of Anatomy and Neuroscience, The University of Melbourne, [2]Department of Physiology, University of Iceland

Adenosine acts as a neurotransmitter at a range of purine (P1) receptors, including A_1, A_{2A}, A_{2B} and A_3. Activation of adenosine receptors is likely to be important for signaling between neurons and glial cells in the retina. The aim of this study was to characterise the location and function of the A_{2A}-R in the rodent retina. The location of the A_{2A}-R was evaluated in the mouse retina using immunohistochemistry. In the outer plexiform layer, punctate A_{2A}-R expression was found associated with rod but not cone photoreceptor terminals and was closely associated with horizontal, rod bipolar, and cone bipolar cells. In the inner retina, A_{2A}-R labeling was observed on calretinin-positive amacrine cells, and rod and cone bipolar cell terminals. A_{2A}-R expression was also observed on glia; Müller cells and astrocytes. The role of the A_{2A}-R in modulating retinal function was assessed in the rat using the electroretinogram (ERG). Intravitreal administration of the A_{2A}-R agonist, CGS21680 [2mM] decreased post-photoreceptor responses, including the rod bipolar cell (scotopic b-wave) (298.2 ± 21.5 to 212.5 ± 19.3 μV; n=6, p=0.005), the cone bipolar cell (photopic b-wave) (124.3 ± 17.7 to 87.8 ± 11.2 μV; n=6, p=0.045) and the inner retinal, amacrine cell response (oscillatory potentials) (99.9 ± 9.4 to 47.2 ± 11.4 μV; p=0.023). ZM241385 [4mM], an A_{2A}-R antagonist did not have any effect on the ERG. Adenosine provides input, via the A_{2A}-R, to neurons and glia in the retina, providing inhibitory input to inner retinal neurons that modulate the visual response.

Poster 187 – Tuesday 6th December

DYNAMIC AND SELF REGULATED FATE SPECIFICATION FOLLOWING RETINAL INTERNEURON ABLATION IN THE ZEBRAFISH

Dr Jeremy CK Ng[2], Professor Peter D Currie[2], **Dr Patricia R Jusuf[1]**

[1]School of Biosciences, University Of Melbourne, [2]Australian Regenerative Medicine Institute, Monash University

Extrinsic cues (e.g. environmental) during development can provide feedback to control the proportion of different neuron types generated, which contributes towards appropriate circuit formation. It is unknown whether these cues also direct regeneration. Here, we assessed whether extrinsic feedback could direct fate specification in a zebrafish regeneration retina model.
Two injury models were established to ablate different retinal neurons. The mechanical needle stick injury affects all retinal neuron types whereas the genetic chemical injury only ablates interneurons expressing the nitroreductase enzyme that converts the harmless drug metronidazole into a cytotoxin in these cells. The subsequent regeneration was characterised histologically with emphasis on the temporal progression and fate of regenerating neurons.
After both injuries, cell death (TUNEL) occurred at 1 – 3 days post injury (dpi) and stem cell proliferation (BrdU/PCNA) primarily occurred in the first 7dpi. However, neurogenesis after the genetic chemical injury was initially significantly biased towards regenerating only the affected cell types (73%) compared to mechanical injury (42%, t-test p-value = 0.006) followed by a dynamic switch to regenerating all cell types as the appropriate neural proportions were restored.
Thus, feedback from surviving neurons in the injured retina can direct the correct fate specification of newly regenerated neurons. This feedback is dynamic and continuously readjusts signals as regeneration proceeds resulting in restoration of neuron type distribution found in the uninjured retina. Such endogenous signals could be integrated in regenerative approaches by supplementing relatively immature neural progenitors or precursors to guide fate specification into desired neurons.

Poster 188 – Monday 5th December

THE ROLE OF PACAP AND ROSTRAL VENTROLATERAL MEDULLARY NEURONS IN SYMPATHETIC ELEVATION FOLLOWING ACUTE-INTERMITTENT HYPOXIA

Ms Zohra Kakall[1,2], Dr Melissa MJ Farnham[1,2], Dr Polina E Nedoboy[1,2], Professor Paul M Pilowsky[1,2]

[1]Department of Physiology, Sydney Medical School, The University of Sydney, [2]Heart Research Institute

Objective: Intermittent hypoxia causes a persistent increase in sympathetic activity, which progresses to hypertension in chronic conditions such as obstructive sleep apnoea. Pituitary adenylate cyclase activating polypeptide (PACAP) is an excitatory neurotransmitter with long-lasting sympathetic effects. Cardiorespiratory regulation occurs in the rostral ventrolateral medulla (RVLM). We aimed to determine whether intermittent activation of the RVLM causes sustained elevation of splanchnic sympathetic nerve activity (sSNA), termed long-term facilitation (LTF), mediated by PACAP.
Methods: The role of PACAP signalling in mediating sympathetic LTF in response to intermittent activation of the RVLM was investigated in urethane-anaesthetized and artificially ventilated rats (n=53 Sprague–Dawley).
Results: Intermittent microinjections of glutamate into the RVLM caused sympathetic LTF (n=5; Δ64.1 ± 11.5%; P< 0.01), compared to PBS control (n = 4; Δ-3.0 ± 2.5%). Significantly, the RVLM-glutamate induced LTF was abolished by prior intrathecal infusion of the PACAP antagonist, PACAP(6-38) (n=4; Δ7.7 ± 15.1%; P< 0.01). Intermittent bilateral disinhibition of the RVLM did not elicit sLTF. Intermittent, unilateral, low dose microinjections of PACAP in the RVLM also did not elicit sLTF. However, bilateral microinjection of PACAP(6-38) in the RVLM blocked the development of AIH-induced sLTF (n=3; Δ-21.8 ± 4.5%).
Conclusion: Our results indicate that the RVLM is a crucial site for the development of sympathetic LTF, and is not solely due to the actions of hypoxia. Our results also suggest that PACAP plays an essential role, within the RVLM in mediating AIH-induced sympathetic LTF, and therefore may contribute to the development of sleep apnoea induced hypertension.

Poster 189 – Tuesday 6th December

ON THE APPLICATION OF SUBSENSORY ELECTRICAL NERVE STIMULATION FOR THE IMPROVEMENT OF VIBRATION PERCEPTION IN PATIENTS WITH HIV RELATED PERIPHERAL NEUROPATHY

Mr David Karpul[1,2], Dr Sarah McIntrye[1,3], Assoc. Prof Jeannine M Heckmann[2], Prof André van Schaik[1], Dr Paul, P Breen[1]

[1]The MARCS Institute, Western Sydney University, [2]Division of Neurology, Department of Medicine, University of Cape Town, [3]Neuroscience Research Australia

Length dependant peripheral neuropathy affects millions of people worldwide. Approximately half of people with HIV in South Africa have peripheral neuropathy (HIV-PN), the effects of which include loss of sensation and various forms of pain. Subsensory Electrical Nerve Stimulation (SENS) is the application of stochastic electric current through surface electrodes at imperceptible amplitudes. This form of therapy has been shown to immediately improve sensation distal to the application site in both healthy participants and elderly participants during application. We applied SENS to the ankle of 12 patients previously diagnosed with HIV-PN and 7 age-matched healthy controls. All participants were recruited in Cape Town, South Africa. We measured sinusoidal vibration thresholds (VPT) at 25 Hz, 50 Hz and 128 Hz under the hallux with a 5 mm spherical probe contacting the skin. We found that vibration frequency influenced VPT ($p<0.05$) but that HIV-PN status did not significantly alter VPT. SENS improved VPT significantly at 50 Hz (mean improvement 18.5 %, $p<0.05$) but not at other frequencies, regardless of HIV status. This may be an artefact of the study design, as the SENS amplitude was optimised for 50 Hz. The finding that HIV-PN status did not influence VPT has significant implications for further research in HIV-PN. Further investigation should be conducted as to why this cohort did not exhibit the response to SENS at all frequencies, as this may elucidate both the mechanism of SENS and the mechanisms of HIV-PN.

Poster 190 – Monday 5th December

GREEN TEA EXTRACT INHIBITS CEREBRAL ISCHEMIA INDUCED OXIDATIVE STRESS, NEUROINFLAMMATION AND APOPTOSIS IN EXPERIMENTAL MODEL OF STROKE IN MICE

Ms Harjeet Kaur[1], Dr Bikash Medhi[1], Dr BD Radotra[2], Dr Manish Modi[3], Dr Amitava Chakrabarti[1]

[1]Pharmacology, PGIMER, [2]Histopathology, PGIMER, [3]Neurology, PGIMER

Abstract: Stroke is one of the major challenges to health and the reason for increasing disability-adjusted life years. Currently, limited treatment options are available and many promising drugs have failed in human clinical trials due to intolerable side effects or therapeutic limitations. The present work was therefore designed to study the effect of green tea extract and progesterone as a neuroprotective agent in the mouse model of partial global cerebral ischemia. The 14 days study protocol was comprised of five groups (n=24 in each group): Sham operated, Surgical operated, Vehicle treated, Progesterone treated, Green tea extract treated groups. The Neurobehaviour tests (Elevated plus size, Grip strength), oxidative stress markers, pro-inflammatory markers, MMPs, apoptosis and histopathology were evaluated at the end of 14 days. Progesterone and Green tea extract have consistently been shown to reduce free radicals production, neuroinflammation and improved behaviour outcome. Histopathological examination indicated that green tea extract and progesterone treatment inhibited neuronal injury induced by cerebral ischemia. These findings strongly implicated the neuroprotective potential of Green tea extract in this experimental model of stroke as evident by amelioration of oxidative stress, inflammation and apoptosis.

Poster 191 – Tuesday 6th December

THE IMPACT OF ACUTE NEUROINFLAMMATION ON INTRINSIC EXCITABILITY OF CHOLINERGIC NEURONS IN THE SEPTUM

Orsolya Kekesi[1,2], Dr. Erika Gyengesi[1], Prof Gerald Muench[1], Dr. Yossi Buskila[2]

[1]Group of Pharmacology, School of Medicine; Western Sydney University, [2]Biomedical Engineering and Neuroscience group, The MARCS Institute; Group of Anatomy and Cell biology, School of Medicine; Western Sydney University

Alzheimer's disease is a neurodegenerative disorder characterized by significant impairment of cognitive function, memory loss and behavioural phenotypes such as anxiety and depression. There are several hypotheses regarding the etiology of A.D. The first and oldest hypothesis is the "cholinergic hypothesis" which stated that a serious loss of cholinergic function in the basal forebrain and the associated loss of cholinergic innervation in the hippocampus and neocortex contribute significantly to the cognitive symptoms associated with AD. However, the source underling the loss of cholinergic cells is still unknown. Recent findings suggest that neuroinflammation is a preliminary process, which play a role in the onset of Alzheimer's disease. However, the impact of neuroinflammation on cholinergic neurons is still an undiscovered area.

In this study we have investigated the impact of acute neuroinflamation on the biophysiological properties of cholinergic neurons in the septo-hippocampal pathway. Acute neuroinflammation was induced by peripherial administration of lipopolysaccharide (200 µg/kg) into ChAT(BAC)-eGFP mice, which express the green fluorescent protein under the promoter of choline acetyl transferase. Following neuroinflamation, both passive and active membrane properties of cholinergic neurons were monitored through single cell electrophysiological measurements.

Our results so far indicate on a significant increase of the fast afterhyperpolarization (*f*AHP) amplitude following acute neuroinflammation. These results are indicative of alterations in intrinsic excitability, which may contribute to the cholinergic loss during AD.

Poster 192 – Monday 5th December

VARIATION IN LEFT POSTERIOR PARIETAL-MOTOR CORTEX INTERHEMISPHERIC FACILITATION FOLLOWING RIGHT PARIETAL CONTINUOUS THETA-BURST STIMULATION IN HEALTHY ADULTS

Mr Christopher Killington, Dr Christopher Barr, Dr Tobias Loetscher, Dr Lynley Bradnam
[1]*Repatriation General Hospital*

Spatial neglect is modelled on an imbalance of interhemispheric inhibition (IHI); however evidence is emerging that it may not explain neglect in all cases. The aim of this study was to investigate the IHI imbalance model of visual neglect in healthy adults, using paired pulse transcranial magnetic stimulation to probe excitability of projections from posterior parietal cortex to contralateral primary motor cortex bilaterally. Motor evoked potentials were recorded from the first dorsal interossei and facilitation was determined as a ratio of conditioned to non-conditioned MEP amplitude. A laterality index reflecting the balance of excitability between the two hemispheres was calculated and a temporal order judgement task assessed visual attention. Continuous theta-burst stimulation was used to transiently suppress right parietal cortex activity and the effect on laterality and judgement task measured, along with associations between baseline and post stimulation measures. Findings indicate there was relative balance between the cortices at baseline. Stimulation had conflicting results on laterality, and right PPC suppression did not evoke left PPC facilitation in most participants, contrary to the IHI imbalance model. Correlation analysis suggests a strong association between laterality direction and degree of facilitation of left PPC-to right M1 following stimulation (r=.902), with larger MEP facilitation at baseline demonstrating greater reduction (r=-.908). Left M1 facilitation prior to stimulation may predict an individual's response to continuous theta-burst stimulation of right PPC.

Poster 193 – Tuesday 6th December

IMPAIRED REINSTATEMENT OF CONDITIONED FEAR IN AGED MICE IS RECOVERED BY CHRONIC EXERCISE

Dr Annabel Short[1,2], Mr Viet Bui[1,2], Dr Heather Madsen[1,2], Professor Anthony Hannan[1,2], **Dr Jee Hyun Kim[1,2]**

[1]*The Florey Institute Of Neuroscience And Mental Health,* [2]*University of Melbourne*

Our aging population has major mental health implications both for the individuals and the society. One serious consequence is the changes in the brain that accompany aging, especially in the hippocampus that is vulnerable to damage throughout life. Fortunately, the hippocampus is dynamic, and chronic exercise can maintain new neurons in the hippocampus. The hippocampus is critical for many types of learning and memory. In particular, cognitive flexibility involved with extinction and reinstatement of conditioned fear requires the hippocampus. Therefore, we asked whether chronic exercise in middle-aged mice can improve extinction and/or reinstatement of conditioned fear compared to standard housing. Eight-months old C57Bl/6J mice either had access to a running wheel or remained in standard housing for three months, until 11-months of age. Then they received tone-footshock pairings, which was subsequently extinguished with tone-alone presentations the next day. Half of the mice then received a reminder treatment in the form of a single footshock. Interestingly, 11-month-old mice housed in standard conditions exhibited impaired reinstatement ($p < 0.05$). That is, the reminder treatment did not recover the extinguished fear compared to mice that did not receive any reminder. This suggests that aged mice display decreased cognitive flexibility. However, the reminder treatment was able to reinstate extinguished fear in 11-month-old mice that had access to a running wheel from 8 months of age ($p < 0.05$). These results show that positive changes in lifestyle may be beneficial in reducing natural decline in cognitive abilities even when the change occurs late in life.

Poster 194 – Monday 5th December

ANALYSIS OF LIPOPROTEIN-ASSOCIATED LIPIDS IN FRONTOTEMPORAL DEMENTIA AND ALZHEIMER'S DISEASE

Dr Woojin Kim[1,2], Eve Jary[1], Russell Pickford[2], Lauren Bartley[1], Olivier Piguet[1,2], John Hodges[1,2], Glenda Halliday[1,2]

[1]*Neuroscience Research Australia,* [2]*University of New South Wales*

Frontotemporal dementia (FTD) is a form of dementia with heterogeneous clinical and pathological presentations. A common sub-type of FTD is behavioural variant FTD (bvFTD). In contrast to Alzheimer's disease (AD), FTD is characterized by considerable early loss of significant amounts of brain tissue with concomitant loss of lipids. However, very little is known about lipid changes in FTD. Here, we undertook a comprehensive analysis of lipids in FTD with the aim of developing lipid biomarkers for FTD. We collected blood from 16 bvFTD, 11 AD and 22 controls without dementia and extracted the plasma lipids using Folch extraction. Extracted lipids were measured using liquid chromatography-mass spectrometry. Relative quantification of lipid species was obtained from peak areas normalised to internal standards. We focused on lipids that are associated with lipoproteins since metabolic abnormality is a key feature of bvFTD. The major plasma glycerolipids – triacylglycerols (TAG) and diacylglycerols (DAG) – were significantly increased in FTD compared to controls. 97 TAG and 32 DAG species were significantly increased in FTD with a mean increase of 84% and 155% respectively. We also analyzed phospholipids – phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine, and they were significantly decreased in FTD. By contrast in AD, both glycerolipids and phospholipids were unaltered. These data indicate significant changes in the circulating plasma lipids in bvFTD and provide evidence for lipoprotein lipid dysfunction in FTD pathology. Furthermore, specific lipid species that could potentially serve as biomarkers to differentiate FTD from AD and people without dementia were identified in our study.

Poster 195 – Tuesday 6th December

INTERMITTENT INTRAVENOUS INJECTIONS OF CIRCULATING ANGIOTENSIN II CAUSES SUSTAINED INCREASES IN SYMPATHETIC NERVE ACTIVITY IN ANAESTHETISED RATS

Mr Seung Jae Kim[1], Dr Melissa Farnham[2], Professor Paul Pilowsky[1], Dr Stephen Abbott[2]

[1]The University Of Sydney, [2]Heart Research Institute

Obstructive sleep apnoea (OSA) is a condition characterised by intermittent hypoxia. Hypoxia increases plasma renin and angiotensin II (AngII) levels, which may contribute to elevations in resting SNA and blood pressure in patients with OSA. The aim of the present study was to determine if intermittent injections of low doses of AngII, analogous to the pattern of hypoxia during acute intermittent hypoxia, causes a sustained elevation of SNA. Sprague-Dawley rats were anaesthetised with urethane (1.3 $g \cdot kg^{-1}$, i.p.), vagotomised, paralysed, artificially-ventilated, and splanchnic and phrenic nerve activities were recorded. Repetitive intravenous injections of AngII (35 pmol x10 in 0.1 ml, 5 min intervals) increased SNA relative to baseline over a 1 hour recording period (52.4 ± 4.5%, n=5), whereas the same total dose of AngII delivered in a single intravenous injection (350 pmol in 0.1 ml) had no effect on SNA (6.5 ± 2.6%, n=4). Similarly, intermittent intravenous injections of vehicle (0.9% NaCl x10 in 0.1 ml) had no effect on SNA (3.0 ± 2.9% from baseline, n=4). These results show that intravenously administered AngII is capable of producing sustained increases in SNA when it is delivered in an intermittent fashion, analogous to acute intermittent hypoxia. This work suggests that intermittent surges in AngII during apnoea contributes to the development of hypertension in OSA.

Poster 196 – Monday 5th December

ACTIVITY-DEPENDENT REGULATION OF A DENDRITIC KINESIN INVOLVED IN LEARNING AND MEMORY

Professor Erika Holzbaur[1]

[1]Perelman School of Medicine, University of Pennsylvania

Neurons are highly polarized cells that depend on active and specific trafficking of cargos to and from axons and dendrites. While the axon is specialized for long distance trafficking from soma to synapse and back, trafficking in dendrites is more complex. Microtubule organization is also more complicated, as dendritic microtubules are oriented with mixed polarity as opposed to the unipolar orientation found in axons. Further, the dendritic cytoskeleton undergoes activity-dependent remodeling during learning and memory. However, the mechanisms that dynamically regulate these processes remain largely unknown. Here, we focus on the dendritic kinesin, KIF21B. Using single molecule assays in vitro and live cell imaging of organelle and microtubule dynamics in primary rat hippocampal neurons, we identified the kinesin-4 motor KIF21B as a key regulator of both activity-dependent trafficking and microtubule dynamicity in dendrites. We find that KIF21B is essential for the retrograde trafficking of BDNF/TrkB complexes, and loss of KIF21B leads to altered BDNF signaling to the nucleus. KIF21B also regulates microtubule dynamics, via a separable C-terminal microtubule-binding domain. Critically, neuronal activity enhances the motility of KIF21B at the expense of effects on microtubule dynamics. Thus, KIF21B is the first example of a kinesin whose function is directly tuned to neuronal activity state. Consistent with these observations, knockout of KIF21B in mice leads to defects in learning and memory (Muhia et al., 2016). Together, these data support a model in which KIF21B navigates the complex dendritic cytoskeleton by differentially regulating motor and microtubule dynamicity functions in an activity-dependent manner.

Poster 197 – Tuesday 6th December

MAINTAINING CONNECTIVITY IN NEURODEGENERATIVE DISEASES OF AGEING

Dr Anna King[1], Dr Nan Tian[1], Ms Rachel Atkinson[1], Ms Kelsey Hanson[1], Mr James Bender[1], Mr Sam Dwyer[1], Dr Jacqueline Leung[1], Professor James Viclers[1]

[1]Wicking Dementia Centre, University Of Tasmania

Neurodegenerative diseases of ageing are characterized by a functional disconnection between regions of the nervous system, which is thought to arise from degeneration of neuronal processes. The overarching aim of our work is to investigate mechanisms of neurite and synapse degeneration and determine protective strategies. Our studies show that proteins pathologically associated with disease, such as TDP-43, have key roles in maintaining neurites. However, pathological conditions such as excitotoxicity also cause neurite loss. Interestingly, these diseases usually do not manifest until later in life, even in the presence of mutant disease-causing proteins. This suggests that alterations during ageing contribute towards the neuronal vulnerability to disease. Therefore in this study we have investigated alterations to the neuronal cytoskeleton, a key conduit of neurite breakdown, during ageing. Cortical and hippocampal tissue was collected from C57Bl/6 mice at 22 months and 10 weeks of age (N=5), and Western blot analysis performed to examine microtubule, neurofilament and associated proteins. Our findings show that whilst post-translational modifications of microtubule proteins were not significantly changed with ageing ($p>0.05$), there were significant ($p<0.05$) alterations to microtubule-associated proteins, including an up-regulation of the plasticity associate protein CRMP2 and phospho-CRMP2 and a down-regulation of tau. Neurofilament expression was also altered. Our ongoing studies are using *in vitro* and *in vivo* techniques to investigate the role of pathological conditions in neurite loss, in the context of ageing. These data suggest that the ageing cytoskeleton may increase the vulnerability of neurons to pathological conditions, and be a target for neuroprotection.

Poster 198 – Monday 5th December

PROLACTIN INTERACTION WITH THE MEDIAN EMINENCE INCREASES PHOSPHO-STAT5 AND REDUCES PERMEABILITY.

Siobhan Kirk[1], Professor Dave Grattan[1], Associate Professor Stephen Bunn[1]

[1]*University Of Otago*

Centre for Neuroendocrinology and Department of Anatomy, University of Otago, Dunedin, New Zealand.

Prolactin, released from the anterior pituitary, is regulated by hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons. When circulating prolactin is elevated, TIDA neurons release dopamine at the median eminence (ME), which inhibits further prolactin release. The objective of this study was to determine if the ME is a target of prolactin action. Mice (C57BL/6 males, n=3-7 per group) were pre-treated with bromocriptine to suppress endogenous prolactin secretion prior to administration of ovine prolactin (2 mg/kg) or saline. Mice were euthanised with pentobarbital 2-60 min later and perfused with 4% paraformaldehyde. Immunohistochemistry demonstrated that prolactin increased phosphorylated signal transducer and activator of transcription 5 (pSTAT5) in the arcuate nucleus and ME. This response was rapid ($p < 0.05$ within 2 min) and observed in nuclear and non-nuclear regions of responding cells. Nuclear pSTAT5 in the ME was primarily localised within tanycytes (dual-labelled with vimentin), whereas non-nuclear pSTAT5 was expressed within neuronal processes (dual-labelled with β-III tubulin). This neuronal location was confirmed by its absence in mice lacking the prolactin receptor in neurons ($p<0.05$). However these did not appear to be neuroendocrine populations of neurons because pSTAT5 staining failed to colocalise with fluorogold (16 h treatment). The ME prolactin response was associated with a decrease in its permeability, as shown by reduced Evans Blue staining (60 min treatment) ($p<0.05$). This response was significantly attenuated in mice lacking neuronal prolactin receptors $p<0.05$). Thus cells within the ME respond to circulating prolactin and this response may result in changes in the accessibility to blood borne factors.

Poster 199 – Tuesday 6th December

ALTERED MICROGLIA DYNAMICS AT THE SITE OF SECONDARY NEURODEGENERATION AFTER STROKE

Murielle Kluge[1]

[1]*University Of Newcastle,* [2]*Hunter Medical Research Institut*

Stroke is one of the leading causes of death and disability worldwide. As the primary immune cells of the brain microglia play a major role in the initial response and inflammation after stroke. Recent research has shown how remarkably motile and dynamic microglia cells are, highlighting the need for a better understanding on how dynamic properties translate to microglia function. We are investigating the dynamic and functional properties of microglia following stroke. Our research is focused on microglial baseline motility and their ability to respond towards locally induced laser damage at both the primary infarction and thalamic secondary neurodegeneration sites. Using a phototrombotic stroke model in mice, in combination with acute brain slice preparation and ex-vivo live cell multi-photon imaging we found differences in microglia process dynamics, 14days after a cortical stroke. This finding is particularly interesting, as both subpopulations of microglia showed an activated morphology and similar baseline motility but differences in there process extension towards a laser damage as well as phagocytic activity. Our results suggest that changes in dynamic properties are correlated to differences in microglia function. This leads to the hypothesis that the activation phenotype of microglia at sites of SND is different from those at the site of acute damage after stroke and therefore might have implications to the nature and progression of SND through-out the brain.

Poster 200 – Monday 5th December

CEREBRAL DISTRIBUTION OF TRACE ELEMENTS IN THE COMMON MARMOSET

Beate Knauer[1], .[2], Dominic J. Hare[3], .[4], Bence Paul[5], Hsin-Hao Yu[6], Kirsty Watkins[6], Daria Malamanova[6], Piotr Majka[7], .[8], Marcello G.P. Rosa[1], .[8], David H. Reser[6]

[1]*Monash University,* [2]*Research School, Ruhr University Bochum,* [3]*Elemental Bio-imaging Facility, University of Technology Sydney,* [4]*The Florey Institute of Neuroscience and Mental Health, The University of Melbourne,* [5]*iolite Software, School of Earth Sciences, The University of Melbourne,* [6]*Department of Physiology, Monash University,* [7]*Nencki Institute of Experimental Biology, Polish Academy of Sciences,* [8]*Australian Research Council, Centre of Excellence for Integrative Brain Function*

Purpose: The trace elements iron, copper, zinc, and manganese are crucial for neuronal viability. Even though trace elements are known to be altered in neurodegenerative diseases their role therein is incompletely understood.
Methods: Using fixed, frozen brains from 4 adult marmosets, we scanned 40 μm thick coronal, sagittal and flat mounted sections of cerebral cortex. Laser ablation-inductively coupled plasma-mass spectrometry scans were performed for Fe, Cu, Zn, and Mn, and aligned with adjacent sections stained for the Nissl, myelin, cytochrome oxidase, and calbindin.
Results: We observed reliably non-uniform distributions of Fe, Mn and Zn across the marmoset brain. Zn concentrations were higher in the white matter than in the cortex. Both Fe and Mn concentrations were higher in the grey than in the white matter and particularly enriched in primary sensory areas. The enrichment of Fe in V1 in comparison to extrastriate areas could be replicated in the flat mount. Individual cytochrome oxidase blobs in the flat mounted V1 could not be differentiated based on trace elements.
Conclusions: To guide future studies on the pathophysiological function of trace elements a comprehensive map of their cerebral distribution in the healthy primate is needed. The marmoset is an ideal model for mapping the distribution of trace elements across an

entire primate brain. This study demonstrates that trace element concentrations can be resolved with respect to anatomical (cyto- and myelo-architectonic) and functional (primary sensory) cortical boundaries with high fidelity and reproducibility.

Poster 201 – Tuesday 6th December

MIR-744 AND MIR-224 REGULATE NEUROGENESIS IN VITRO THROUGH DOWNREGULATION OF NPAS4

Professor Simon Koblar[1], Dr Fong Chan Choy[1], Dr Thomas Klaric[1], Dr Martin Lewis[1]

[1]University Of Adelaide, SAHMRI

Neuronal PAS domain protein 4 (Npas4) is a brain-specific transcription factor whose expression is enriched in neurogenic regions of the brain. We have demonstrated in vitro that Npas4 expression is dynamic and highly regulated during neural differentiation of embryonic stem cells (ESCs). While these findings implicated a role for Npas4 in neurogenesis, the underlying mechanisms of regulation remained unknown. Given that growing evidence suggests that microRNAs (miRNAs) play important roles in both embryonic and adult neurogenesis, we reasoned that miRNAs might be good candidates for regulating Npas4 expression during neural differentiation of ESCs. In this study, we utilized RNA-Seq to profile miRNA expression during neural differentiation of mouse ESCs. Two differentially expressed miRNAs were identified to be able to significantly reduce reporter gene activity by targeting the Npas4 3'UTR, namely miR-744 and miR-224. More importantly, ectopic expression of these miRNAs during neural differentiation resulted in downregulation of endogenous Npas4 protein expression. Functional analysis revealed that overexpression of either miR-744 or miR-224 delayed early neural differentiation, reduced GABAergic neuron production and inhibited neurite outgrowth. Collectively, our findings indicate that Npas4 regulates neuronal differentiation.

Poster 202 – Monday 5th December

TNF-α IN THE AREA POSTREMA ELICITS SYMPATHETIC EXCITATION TO THE HEART IN RATS

Dr Willian Korim[1], Jeremy Basser[1], Kahlid Elsaafien[1], Dr Song Yao[1]

[1]Florey Institute Of Neuroscience And Mental Health

Neuroinflammation is a consequence of high levels of circulating cytokines commonly observed in heart failure. A leading mediator of this response is tumor necrosis factor alpha (TNF-α), the receptors for which are expressed throughout the area postrema - a circumventricular organ found in the dorsal medulla. In this study we sought to determine whether activation of TNF-α receptors type 1 (TNFR1) in the area postrema increases the sympathetic drive to the heart, contributing to the detrimental effects in heart failure. In anesthetized male Sprague-Dawley rats, we show that TNF-α increases the sympathetic nerve activity to both the heart and blood vessels in the periphery. Microinjection of TNF-α augmented ($P<0.01$, N=5) cardiac (+53±12%) and renal (+34±7%) sympathetic nerve activities, resulting in rises (N=7) in arterial blood pressure (Δ: +8.3±3.7 mmHg, $P<0.05$) and heart rate (Δ: +27.1±9.2 bpm, $P<0.01$). All responses were attenuated by prior microinjection of TNFR1 antibody in the same site. Together the results suggest that activation of TNFR1 in the area postrema enhances the detrimental hemodynamic effects in heart failure by predominantly increasing cardiac sympathetic nerve activity.

Poster 203 – Tuesday 6th December

NEUROPROTECTIVE POTENTIAL OF ZONISAMIDE IN MES INDUCED SEIZURES

Mr Baldeep Kumar[1], Dr Bikash Medhi[1], Dr Manish Modi[2], Dr Biman Saikia[3]

[1]Department of Pharmacology, Postgraduate Institute of Medical Education and Research, [2]Department of Neurology, Postgraduate Institute of Medical Education and Research, [3]Department of Immunopathology, Postgraduate Institute of Medical Education and Research

Abstract: Epilepsy is a chronic neurological condition characterized by recurrent seizures, almost affects people in every country throughout the World. Recently, the neuroscientists and neurologists have investigated the use of antiepileptic drugs to prevent neuronal loss and the cognitive impairment which is commonly seen with the progression of epilepsy. In this study, we evaluated the neuroprotective mechanism of zonisamide in MES induced seizures in rats. MES induced seizures lead to increased oxidative stress and activation of neuroinflammatory pathway. Our results indicated that treatment with zonisamide showed a significant reduction in seizure activity, decreased the lipid peroxidation and ameliorated the oxidative stress induced by MES seizures. The inflammatory processes in the brain contribute to the etiopathogenesis of seizures and epilepsy and this is increasingly recognized as a result of supportive evidence in experimental models and in the clinical setting. In this study, we found a significant increase in inflammatory mediators after MES seizures. However, the administration of zonisamide abolished the activation of neuroinflammatory cytokines. Together, these findings indicated the neuroprotective potential of zonisamide by preventing oxidative neuronal damage and activation of brain inflammatory mediators in seizures.

Poster 204 – Monday 5th December

TASK-2 AND GPR4 PROVIDE INDEPENDENT CONTRIBUTIONS TO THE CENTRAL VENTILATORY CHEMOREFLEX

Dr Natasha Kumar[1], Dr Yingtang Shi[2], Dr Keyong Li[2], Mr Josh Sen[2], Ms Rosalie James[2], Professor Douglas Bayliss[2]

[1]Department of Pharmacology, UNSW Australia, [2]Department of Pharmacology, University of Virginia

Central respiratory chemoreceptor neurons in the retrotrapezoid nucleus (RTN) of the ventral brainstem express the transcription factor Phox2b and are intrinsically activated by protons. Acidification inhibits a resting potassium conductance, and in about half these cells, this appears to be entirely mediated by the two-pore domain potassium (K2P) channel TASK-2. Recently, we demonstrated that GPR4, a proton-activated G protein-coupled receptor, is highly and selectively expressed by RTN neurons (Kumar *et al.* 2015, Science). Furthermore, GPR4 contributes to pH sensitivity of RTN neurons in vivo. Here, our objective was to characterise the ventilatory phenotype of GPR4/TASK-2 double knockout mice. Using fluorescence in situ hybridisation (RNAscope technology) we also sought to determine whether GPR4 and TASK-2 are co-localized in RTN neurons. In brainstem slices from wild-type mice, GPR4 and TASK-2 were expressed in overlapping populations of Phox2b-expressing RTN chemosensory neurons. However, although 60% and 70% of RTN neurons expressed GPR4 or TASK-2 respectively, only 55% of RTN neurons expressed both TASK-2 and GPR4 (n=3). In GPR4/TASK-$2^{-/-}$ mice, the numbers and rostrocaudal distribution of RTN neurons was preserved. Using whole body plethysmography we found that that simultaneous deletion of GPR4 and TASK-2 nearly eliminates the ventilatory response to CO2. GPR4/TASK-$2^{-/-}$ (n=10) exhibited an 87% decrease in the ventilatory response to CO2, compared to genotype control (GPR4/TASK-$2^{+/+}$; n=10). Together, our data suggest that despite expression in overlapping populations, the molecular sensors GPR4 and TASK-2 provide independent mechanisms for CO_2/H^+ sensing in RTN neurons.

Poster 205 – Tuesday 6th December

PHOSPHORYLATION OF GLUTAMATE RECEPTOR IN VIVO INDUCED BY CHRONIC TREATMENT OF MONOSODIUM GLUTAMATE (MSG)

Mr Mantosh Kumar[1], Dr. M Mayadevi[1], Dr. RV Omkumar[1]

[1]Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram

Excessive activity of Ca2+ channels leads to excitotoxicity in neurons which is one of the causes of cell death in various neurodegenerative diseases. Molecular mechanisms leading to cell death in excitotoxic conditions are still not completely understood. Monosodiumglutamate (MSG) is known to cause excitotoxicity *in vivo* . In this study we are observing the changes at the level of protein expression as well as their post translational modification under excitotoxic conditions in the MSG treated rat model system. Activation of calcium signalling via glutamate receptor is expected upon chronic treatment with MSG. Briefly, methodology includes 15 days of MSG injection via i.p route in 100-150 gm adult male rat, which were given orally either vehicle or an NMDAR inhibitory plant extract. A group of animals were also fed with a known NMDAR inhibitory drug dextromethorphan. Analysis by Morris water maze (MWM) test showed that the behavioural impairment caused by MSG administration could be ameliorated by simultaneous treatment with one of the NMDAR inhibitors, either the plant extract or dextromethorphan. Increased p-GluN2B was observed by immunoblotting in hippocampal and cortical tissues in MSG treated animals which were found to be attenuated in the group fed with NMDAR inhibitory plant extract. p-GluA1-Ser831 also appears to increase upon MSG treatment. Changes in the levels of, other proteins related to calcium signalling and cell death such as GluN2B, BDNF, p-PP1 and Bcl2 are also being analysed . Data from this study might provide new insights on signaling pathways during excitotoxiciy.

Poster 206 – Monday 5th December

IMPAIRED GABA SIGNALLING IN THE HUMAN ALZHEIMER`S DISEASE BRAIN

Dr Andrea Kwakowsky[1], Tessa Fuhrer[1], Associate Professor Henry Waldvogel[1], Dr Beth Synek[1,2], Dr Clinton Turner[1,2], Professor Richard Faull[1]

[1]Centre for Brain Research, Department of Anatomy and Medical Imaging, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand, [2]Department of Anatomical Pathology, LabPlus, Auckland City Hospital, Auckland, New Zealand

Alzheimer's disease (AD) is a neurodegenerative disorder that affects millions of people worldwide. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and plays an important role in regulating neuronal excitability. GABA reuptake from the synapse is dependent on specific transporters - mainly GAT-1, GAT-3 and BGT-1 (GATs). This study is the first to show alterations in the expression of the GATs in the Alzheimer's disease hippocampus, entorhinal cortex and superior temporal gyrus using immunohistochemistry and confocal microscopy. We found a significant increase in BGT-1 expression associated with AD in all layers of the dentate gyrus, in the stratum oriens of the CA2 and CA3 and the superior temporal gyrus. In AD there was a significant decrease in GAT-1 expression in the entorhinal cortex and superior temporal gyrus. We also found a significant decrease in GAT-3 immunoreactivity in the stratum pyramidale of the CA1 and CA3, the subiculum and entorhinal cortex. These findings indicate that the expression of the GATs shows brain region and layer specific alterations in AD, suggesting a complex activation pattern and possibly co-regulation of different GATs during the course of the disease. (188 words).

We thank the families of patients who supported this research through their donation of brains to the New Zealand Neurological Foundation Human Brain Bank**.** This work was supported by the Aotearoa Foundation, Centre for Brain Research, University of Auckland, the Health Research Council of New Zealand, Otago Medical School and the Department of Physiology, University of Otago.

Poster 207 – Tuesday 6th December

INDUCTION OF COLONIC MOTOR ACTIVITY USING OPTOGENETICS

Dr Jing Feng[1], Dr Jialie Luo[1], Dr Tim Hibberd[2], **Miss Melinda Kyloh[2]**, Dr Pu Yang[2], Dr Hongzhen Hu[1], Professor Nick Spencer[2]

[1]Washington University, [2]Flinders University of South Australia

Optogenetics is an exciting new technique to control specific neural pathways in the central nervous system, but has not been demonstrated to modulate the enteric nervous system. In this study, we generated a novel transgenic mouse using Cre-driven expression of the light-gated cation channel, channelrhodopsin-H134R (ChR2-H134R). To do this, mice expressing Cre-recombinase under the calretinin (CAL) promotor were crossbred with Rosa-CAG-LSL-ChR2(H134R)-eYFP-WPRE homozygotes. Resulting CAL-ChR2(H134R) mice expressed the ChR2(H134R)-eYFP fusion gene in CAL neurons. Immunohistochemical analysis of colonic myenteric neurons revealed 97% of CAL-immunoreactive neurons were ChR2(H134R)-eYFP$^+$. CAL-immunoreactive neurons were predominantly cholinergic excitatory neurons. Of all myenteric ChR2(H134R)-eYFP$^+$-neurons, 94% were ChAT-immunoreactive and 6% NOS-immunoreactive. Conversely, ChR2(H134R)-eYFP$^+$-neurons represented 74% of all ChAT-immunoreactive- and 7% of NOS-immunoreactive-neurons (n=3). Mechanical recordings were made from intact whole colons in vitro (n=3). Both CAL-ChR2(H134R) mice and wild-type littermates showed ongoing propagating colonic motor complexes (CMCs, mean interval 280±37s, n=3), which were always blocked by tetrodotoxin. Focal illumination (1-5Hz, 10-60s) of the proximal, mid or distal colon evoked premature CMCs in CAL-ChR2(H134R) mice (mean latency from previous contraction 104±47s, p=0.006, n=3), but not in the wild-type littermates. Tetrodotoxin prevented optogenetic activation of CMCs (7/7 times tested, n=3). In conclusion, we generated transgenic mice specifically expressing opsins in a major neurochemical class of enteric neurons with high efficacy. We provide the first demonstration that focal illumination of a specific class of neuron in the enteric nervous system can evoke propagating CMCs. This study demonstrates that optogenetics could prove a very exciting technique to modulate gastrointestinal transit *in vivo*.

Poster 208 – Monday 5th December

UNDERSTANDING THE EFFECTS OF IRON ACCUMULATION AFTER TRAUMATIC INJURY IN THE HUMAN BRAIN

Linh Q. Lam[1,2], Tony Frugier [2], Bruce X. Wong[1], Ashley I. Bush[1], James A. Duce [1,3,] & Peter J. Crack [2]

[1] Oxidation Biology Unit, The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia
[2] Neuropharmacology Unit, Department of Pharmacology and Therapeutics, The University of Melbourne, Parkville, Victoria, Australia
[3] School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, West Yorkshire, United Kingdom

Abstract
Traumatic brain injury (TBI) represents a major health and socioeconomic burden. Our recent work, suggests that iron is elevated independently of brain hemorrhage. Indeed, a modified perl's technique detects intracellular non-heme iron accumulation in the cortex of TBI-affected post-mortem human brains. This first time observation, to our knowledge, suggests that elevated iron observed after TBI may be a direct consequence of a dysfunctional iron regulatory system. Our previous human data on ceruloplasmin (Cp) and amyloid precursor protein (APP) supports a neuroprotective role by reducing oxidative damage through iron regulation (Ayton et al., 2014). Using the same human cohort, we further characterized the iron regulatory system by measuring expression of transferrin, transferring receptor, ferroportin, ferritin, iron-regulatory protein 1 and 2. Surprisingly, both ferritin (Ft) (~50%, *p<0.05) and ferroportin (Fpn) (~20%, **p<0.01) were down regulated compared to the control group after TBI despite elevated iron concentration as detected using inductively coupled plasma mass spectrometry. Ft and Fpn are involved in the storage and efflux of iron respectively and imply that dysregulation of Ft and Fpn may contribute to the deleterious effects of iron insult after TBI. Findings illustrate a complexity in the iron regulatory system that must be homeostatically controlled for maximum neuroprotection after TBI. Understanding these homeostatic mechanisms will enable the development of new therapeutic approaches to combat iron-induced neurotoxicity in TBI.

Ayton, S., Zhang, M., Roberts, B.R., Lam, L.Q., Lind, M., McLean, C., Bush, A.I., Frugier, T., Crack, P.J., Duce, J.A., 2014. Ceruloplasmin and beta-amyloid precursor protein confer neuroprotection in traumatic brain injury and lower neuronal iron. Free Radic Biol Med 69, 331-337

Poster 209 – Tuesday 6th December

TROPHIC AGENTS FOR PROPHYLAXIS OF CHEMOTHERAPY-INDUCED NEUROPATHY

Dr Lila Landowski[1,2], Professor James Vickers[1], Emeritus Professor Adrian West[2], Professor Bruce Taylor[3,4]

[1]Wicking Dementia Research & Education Centre, [2]School Of Medicine, University Of Tasmania, [3]Menzies Institute for Medical Research, [4]Royal Hobart Hospital

Chemotherapy-induced neuropathy (CIN) is a major limitation of chemotherapy treatment, with permanent symptoms persisting in 40% of cancer survivors, which can significantly compromise quality of life. Patients experience symptoms which can include sensory ataxia, neuropathic pain, allodynia, and numbness. Biopsies indicate that epidermal nerve fibre (ENF) degeneration and loss is a key event in the development of the syndrome and is correlated with changes in measures of mechanical allodynia and thermal hyperalgesia. Given that growth factors promote survival and maintenance of neurons, it has led to the therapeutic application of growth factors *in vivo*; however, this work is fraught with unwanted side-effects and challenges. We propose the use of an alternative trophic factor which appears to act preferentially on injured or stressed neurons. Our group has discovered that metallothioneins (MT), and synthetic peptide derivative Emtin B, can promote growth, survival, and axon pathfinding of sensory neurons *in vitro* and *in vivo*. In an animal model of paclitaxel-induced

CIN, MT or EmtinB was delivered intraperitoneally as a prophylactic agent, at the time of chemotherapy administration. Because of the documented differences in pain processing between males and females, we tested both sexes. There was no protection from mechanical allodynia afforded by the application of MT or Emtin B in female rats. However, in males, modest levels of protection were noted in MT-treated neuropathic rats (32.7g ± 5.5) compared to vehicle treated neuropathic rats (15.2g ± 3.3, p<0.006, *c.f.* non-neuropathic rats, 60.0 ± 0). Further outcome measures will be examined.

Poster 210 – Monday 5th December

RESPIRATORY TRAINING WITH INTERMITTENT HYPERCAPNIA TO IMPROVE DIAPHRAGM FUNCTION FOLLOWING SPINAL CORD INJURY

Dr Michael Lane

Cervical level spinal cord injury (SCI) frequently leads to severe respiratory dysfunction due to damage of the spinal phrenic motor system which controls the diaphragm. While spontaneous functional plasticity does occur following cervical SCI, the extent is limited and diaphragm paresis persists. The goal of this research was to determine if respiratory plasticity and diaphragm recovery could be therapeutically enhanced using a respiratory training strategy: daily acute intermittent hypercapnia (dAIHc). We hypothesized that increasing respiratory activity with dAIHc will stimulate phrenic neuroplasticity and induce respiratory recovery following mid-cervical contusion injury in the adult rat. Anatomical and physiological changes following treatment where investigated using the transynaptic retrograde pseudorabies virus (PRV) tracer which was applied onto the diaphragm ipsilateral to injury to trace phrenic circuitry and terminal diaphragm electromyography (diaEMG) respectively. Western blot and immunohistochemistry were performed to assess changes in plasticity using serotonin (5-HT) and BDNF rostral and caudal to injury. Trained animals showed an increased BDNF expression within the medulla, and greater density of serotonergic axons within the spinal cord when compared with untreated control animals. Ongoing studies are assessing the extent of functional plasticity within dAIHc trained animals. These results suggests that dAIHc is able to promote plasticity within the phrenic network following cervical SCI.

Poster 211 – Tuesday 6th December

A NOVEL WNT SIGNALING PATHWAY REGULATES NEURONAL MORPHOGENESIS

Dr. Vanessa Lanoue[1], Ms Lilly Fogg[1], Ms. Amanda White[1], Mr. Michael Langford[1], Associate Professor Helen Cooper[1]

[1]Queensland Brain Institute

The Wnt molecules play important roles in the development of the brain and the formation of a functional neuronal network. The correct formation of the neuronal dendritic tree is essential for the normal functioning of the nervous system. Abnormalities in dendrite morphology are observed in neurodevelopmental diseases such as autism and schizophrenia. The Wnt receptor Ryk is known for its roles in axonal guidance. It is localized to growth cones and a recent study from our laboratory has suggested that it plays an important role in the control of dendrite growth and branching in the neocortex. We therefore tested this hypothesis using loss- and gain-of-function approaches in primary hippocampal and cortical neuron cultures. Using a RNA interference strategy to knockdown the expression of Ryk we show that Ryk downregulation leads to a significant increase in dendrite growth and branching (total length: shRyk: 848.3 µm ±38.30 µm vs shCo: 615.0 µm ±26.16 µm – p<0.001; total number of dendrites: shRyk: 23.28 ±0.944 vs shCo: 17.67 ±0.715 – p<0.001). Therefore, our data strongly suggest that Ryk plays a key role in determining dendrite morphology in the neocortex and the hippocampus. Currently, we are investigating the identity of the Wnt ligands responsible for Ryk activity in these neurons. These studies will provide a better understanding of the molecular signalling pathways controlling dendritogenesis and the formation of a functional neuronal network.

Poster 212 – Monday 5th December

GENDER-SPECIFIC RELATIONSHIPS BETWEEN HIPPOCAMPAL VOLUME, BDNF GENOTYPE AND BDNF BLOOD LEVELS, IN HEALTHY CONTROLS AND PEOPLE WITH SCHIZOPHRENIA

Ms Cynthia Haidee Lee[1,2], Professor Cynthia Shannon- Weickert[1,2,3], Dr Ashley Skilleter, Professor Rhoshel Lenroot[1,2,3], Professor Peter Buckley[4], Associate Professor Thomas Weickert[1,2,3]

[1]The University of New South Wales, [2]Neuroscience Research Australia, [3]Schizophrenia Research Institute, [4]Georgia Regents University

Brain-derived neurotrophic factor (BDNF) is a potential biomarker for schizophrenia, in part because it plays a critical role in neuroplasticity, is often decreased in the brains and blood of people with schizophrenia and because functional polymorphisms of the human BDNF gene impact cognition and brain volume in healthy humans. This study aimed to determine the extent to which peripheral BDNF levels differ in males and females with schizophrenia compared to controls and test the extent to which BDNF val66met genotype predicts cognitive abilities and brain volume. We measured circulating plasma protein BDNF levels by ELISA, BDNF val66met genotype by PCR based allele discrimination assay, cognitive abilities via tests of verbal memory, language, working memory, processing speed and perceptual organisation, and structural brain volume by MRI in 97 people with schizophrenia and 87 healthy controls. Four brain volume regions of interest were chosen for analysis based on their known relationship with BDNF and cognitive deficits in schizophrenia. We found a significant elevation in plasma BDNF levels in females with schizophrenia, suggesting a gender-specific difference in BDNF levels in people with schizophrenia. Plasma BDNF levels did not significantly predict cognitive performance in either group; however, plasma BDNF positively, and significantly, predicted hippocampal volume in healthy females. The BDNF val-met polymorphism influenced performance

on the language domain across healthy controls and people with schizophrenia, such that met carriers performed better than val-homozygotes. This study reveals complex associations among plasma BDNF, BDNF val66met polymorphism, and brain volume in people with schizophrenia and healthy controls.

Poster 213 – Tuesday 6th December

ALTERED EXPRESSION OF LAMININS POTENTIALLY CONTRIBUTES TO THE PROGRESSION OF DISEASE STATES OF AMYOTROPHIC LATERAL SCLEROSIS

Ms Kah Meng Lee[1], Dr Kirat Chand[1,2], Dr John Lee[1,2], Dr Nickolas Lavidis[1], Dr Massimo Hilliard[3], Dr Peter Noakes[1,3]

[1]School Of Biomedical Sciences, The University of Queensland, [2]Centre for Clinical Research, The University of Queensland, [3]Queensland Brain Institute, The University of Queensland

Amytrophic lateral sclerosis (ALS) is a neurodegenerative disease that is characterised by the loss of motor neurons and degeneration of the neuromuscular junction (NMJ). Transactive DNA binding protein 43 (TDP43) has been shown to be involved in the pathological disease of ALS. TDP43 functions in mRNA processing by transporting and splicing of mRNA transcripts, and has been found to be expressed at the presynaptic nerve terminal of the NMJ, suggesting a potential role of mRNA processing at the NMJ itself. Of note, studies have found that TDP43 interacts with mRNA of laminin chains. Interestingly, absent expression of the laminin-α4 chain at NMJs of hind-limb muscles in human ALS donors has been reported suggesting a potential link between altered laminin chains expression and progression of NMJ degeneration in ALS disease. The present study functionally and morphologically characterised the $TDP43^{Q331K}$ mutation and subsequently aimed to determine the potential involvement of laminins in the mouse model of ALS, $TDP43^{Q331K}$ at 3 months and 10 months utilising immunofluorescence technique. Results showed perturbed transmission properties at 3 month $TDP43^{Q331K}$ NMJs; lower frequency of spontaneous release, decreased quantal content and higher intermittence, which are suggestive of presynaptic defects. Altered innervation patterns; polyinnervation, partial denervation, thin axons and swollen axons were observed at these NMJs at 3 months. Importantly, these changes in both functional transmission and morphological structure at $TDP43^{Q331K}$ NMJs coincided with the mislocalised and missing laminin-α4 chain expression, suggesting the potential involvement of laminin-α4 in the degenerative defects of the NMJ in ALS disease.

Poster 214 – Monday 5th December

QUANTIFICATION OF ROD PATHWAY NEURONS IN THE HUMAN RETINA

Doctor Sammy Chi Sam Lee[1], Rhian Jessi Aghajani[1], Professor Paul Ronald Martin[1], Associate Professor Ulrike Grünert[1]

[1]Save Sight Institute - University Of Sydney

Purpose: The rod pathway involves rod photoreceptors converging onto rod bipolar cells (RBC), which in turn converge onto AII amacrine cells. AII cells are a target for optogenetic restoration of vision in retinal disease, therefore it is important to understand the distribution and density of AII cells and their connections in the retina.
Methods: *Post mortem* donor human eyes (n = 2) were obtained from the Lions NSW Eye Bank at Sydney Hospital and cut at 100 □m thickness. Sections were double labeled for calretinin and GAD to estimate AII cell proportion. Other sections were processed for immunofluorescence to label rods, RBCs, and AII cells. The densities of rods, RBCs, and AII cells were determined from 0 to ~14 mm for one eye. A second eye was quantified for RBCs and AII cells from 0 to ~2 mm.
Results: Rod peak density is at 3 mm with ~150,000 cells/mm^2. Rod density declines to ~80,000 cells/mm^2 at 13 mm. RBC peak density is at 2.5 mm with ~15,000 cells/mm^2. RBC density falls to ~6500 cells/mm^2 at 10 mm. AII cells make up 72% of calretinin-positive cells at ~1 mm and this proportion increases to 93% by 7 mm. AII cells have peak density at 1.5 mm with ~6,600 cells/mm^2 and density declines to ~1,500 cells/mm^2 at eccentricities > 6 mm.
Conclusion: We found convergence from rods to RBCs to AII cells throughout the retina. Our results indicate rod pathway spatial resolution would be limited by the peak density of AII cells.

Poster 215 – Tuesday 6th December

ACUTE MK801 TREATMENT INDUCES WORKING MEMORY DEFICIT IN AUTOMATED TOUCHSCREEN CHAMBERS

Jaime Lee[1], Matthew Hudson[1], Alyssa Sbisa[2], Maarten van den Buuse[3], Jess Nithianantharajah[2], Nigel Jones[1]

[1]Department of Medicine, University Of Melbourne, [2]The Florey Institute of Neuroscience and Mental Health, [3]School of Psychology and Public Health, La Trobe University

Working memory deficits are a consistent cognitive impairment in schizophrenia patients. Current available treatments do not improve these deficits, and this may attributable to our limited understanding of the underlying causal mechanisms. Neural oscillations in the gamma frequency range (i.e. 30-80Hz) are associated with higher order cognitive function, including working memory processes, and the regulation of these high frequency oscillations is impaired in schizophrenia patients. NMDA receptor hypofunction is a strong candidate mechanism involved in the pathophysiology of schizophrenia. Administration of NMDA receptor antagonists can induce aberrant gamma oscillations. Therefore, here we investigate whether disruptions to gamma oscillations induced by NMDA receptor antagonism is related to working memory deficits.

Long Evans rats (N=6) were surgically implanted with depth recording electrodes into the prefrontal cortex and hippocampus. Animals were trained to perform the Trial Unique Non-Matching to Location (TUNL) task of working memory in automated touchscreen chambers. When steady state performance was achieved, they were injected with either saline or MK801 (0.06mg/kg), and electrophysiological recordings were simultaneously performed during working memory behavioural trials of varying levels of difficulty.
Rats performed significantly poorer in the more taxing working memory task (i.e. 10 second delay compared to 1 second delay; $p<0.01$). In addition, treatment with MK801 significantly impaired working memory regardless of task difficulty ($p<0.01$). No differences in performance were observed in trials when the electrophysiological recordings were not conducted. Preliminary analysis suggests that the degree of aberrant gamma oscillations is associated with performance.
These results demonstrate that NMDA receptor hypofunction causes impairment in an advanced working memory paradigm, and suggest that these deficits may be driven by dysregulation of cortical gamma oscillations.

Poster 216 – Monday 5th December

DEVELOPING PHYSIOLOGICALLY RELEVANT MODELS OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Dr Justin Lees[1], Ms Munawwar Abdulla[1], Mr Preet Makker[1], Mr Ryan Tonkin[1], Dr Susanna Park[2], Dr David Goldstein[3], Dr Gila Moalem-Taylor[1]

[1]School of Medical Sciences, The University of New South Wales, [2]Central Clinical School, The University of Sydney, [3]Department of Medical Oncology, Prince of Wales Hospital

The past five decades have seen a substantial increase in the number of people surviving cancer following treatment with chemotherapeutics. In addition to their anti-tumour activity many chemotherapeutics such as platinum based drugs and taxanes have neurotoxic properties. The resulting condition of chemotherapy-induced peripheral neuropathy (CIPN) is an increasingly problematic survivorship issue. Recent clinical trial meta-analysis indicated that there are no currently available neuroprotectants useful in the treatment of CIPN. 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 indicating the presence of neuropathic pain symptoms. In the oxaliplatin model, the treated mice demonstrated significant weight reduction, reduced exploratory behaviour and multiple changes in cytokine expression in the spleen. In paclitaxel-treated mice, we observed thermal hyperalgesia and increased expression of IL-6 and TNF-α in the spleen, but we did not see any difference in weight gain or changes in exploratory behaviour. In both models, we found significant changes in phosphorylation of the heavy neurofilament in the sciatic nerve, which is indicative of distal axonopathy. The results suggest that these models are suitable for testing potential neuroprotectants for the treatment of chronic CIPN.

Poster 217 – Tuesday 6th December

KNOCKDOWN OF PIWI-LIKE PROTEINS IN THE MOUSE HIPPOCAMPUS ENHANCES THE MEMORY OF CONDITIONED FEAR

Ms Laura Leighton[1], Dr Wei Wei[1], Dr Vikram Ratnu[1], Ms Jenny Wang[1], Dr Timothy Bredy[1,2]
[1]Queensland Brain Institute, The University of Queensland, [2]Department of Neurobiology and Behavior and Center for the Neurobiology of Learning and Memory, University of California Irvine

Piwi-interacting RNAs (piRNAs) are a unique class of small regulatory RNAs which interact specifically with the Piwi-like proteins. Through this interaction, piRNAs can modulate gene expression via RNA interference and epigenetic mechanisms. The Piwi pathway has been defined by its role in transposon control during spermatogenesis in mammals, and despite an increasing number of studies demonstrating its expression outside the testes, relatively little is known about its function in mammalian somatic tissues. We have discovered that the Piwi-like proteins Piwil1 and Piwil2 are expressed in neurons throughout the mouse brain, and Piwil2 is upregulated by neuronal activation. Simultaneous knockdown of Piwil1 and Piwil2 in the mouse hippocampus enhances fear learning and the memory of conditioned fear 24 hours later, without affecting generalised anxiety. Preliminary results from small RNA sequencing indicate that some hippocampally-expressed piRNAs are responsive to the fear conditioning paradigm, suggesting that the function of Piwil2 in neurons occurs through piRNA-mediated epigenetic control of transposons or protein-coding genes. Our laboratory is performing FRIP-seq (formaldehyde-crosslinked RNA immunoprecipitation and sequencing) to confirm the interaction between Piwil2 and putative neuronal piRNAs and investigate the regulatory targets of the Piwi pathway in the mammalian brain.

Poster 218 – Monday 5th December

PATHOLOGICAL MUTANTS IN PARKINSON'S DISEASE: WHAT DO THEY TELL US ABOUT ALPHA-SYNUCLEIN'S AGGREGATION PATHWAYS?

Mr Andre Leitao[1], Dr Emma Sierecki[1], Dr Yann Gambin[1], Mr Akshay Bumkhar[1]

[1]University Of New South Wales - EMBL Australia Node in Single Molecule Sciences

Misfolding and pathological aggregation of proteins is a hallmark of many neurodegenerative diseases. α-synuclein (α-Syn) is one of the major components of the Lewy bodies associated with Parkinson's disease and other neurodegenerative disorders called synucleinopathies. Mutations in the SNCA gene were the first reported links between familial sporadic Parkinson's disease and perturbations at the molecular level.

We compared the behavior of α-synuclein and five pathological mutants (A30P, E46K, H50Q, G51D and A53T). To gain insights into the aggregagtion behavior of these proteins, we developed a method coupling single molecule detection and cell-free expression to measure precisely the oligomerisation of proteins, without purification and denaturation steps, in completely undisturbed samples. In these conditions, α-Syn oligomerisation and aggregation is a rapid process that occurred co-translationally, at nM concentrations.
Surprisingly, the pathogenic mutants segregated into two classes: one group forms large aggregates and fibrils while the other tends to form smaller oligomers and fewer fibrils. Strikingly, co-expression experiments reveal that members from the different groups tend to not interact with each other, both at the fibril and monomer levels. Further biochemical analyses revealed differences of structure between the aggregates. Therefore, the different mutants could provide access to different species formed along the fibrillation path of α-Syn.
We then examined the effects of a variety of chaperones on the aggregation propensity of the different mutants. This uncovers the specificty of the different chaperones for specific species in the aggregation pathway and identifies new therapeutic targets in Parkinson's disease and multiple sceloris atrophy (MSA).

Poster 219 – Tuesday 6th December

LOCALISATION OF THE CORTICOSPINAL TRACT IN PIGS: IMPLICATIONS FOR MODELLING TRAUMATIC SPINAL CORD INJURY TO IMPROVE TRANSLATION

Dr Anna Leonard[1,2], Dr Joshua Menendez[4], Dr Betty Pat[2], Dr Mark Hadley[4], Prof Robert Vink[3], A/Prof Candace Floyd[2]

[1]Translational Neuropathology Laboratory, The University Of Adelaide, [2]Department of Physical Medicine & Rehabilitation, The University of Alabama at Birmingham, [3]Division of Health Sciences, The University of South Australia, [4]Department of Neurosurgery, The University of Alabama at Birmingham

INTRODUCTION: Spinal cord injury (SCI) researchers have predominately utilized rodents for SCI modelling and experimentation. Unfortunately, the large number of developed novel treatments for SCI using rodent models have failed to demonstrate efficacy in human clinical trials. Recently, porcine models of SCI have been identified as a valuable intermediary model for preclinical evaluation of promising therapies to aid clinical translation. However, the localisation of the major spinal tracts in pigs has not yet been described. Determining the similarity of the location of the corticospinal (CST), tract in pigs compared to humans may therefore provide important evidence for the use of pigs as a vital pre-clinical model. Therefore, we aim to investigate the localisation of the CST within the porcine spinal cord and determine the similarity to human and rodent anatomy. METHODS: Mature female domestic pigs (n=8, 60kg) received microinjections of fluorescent dextran tracers (Alexa Fluor, 10,000 MW, Life Sciences) into the primary motor cortex to label the CST guided by a STEALTH navigation stereotactic system. At 5 weeks post-injection, animals were euthanized, the entire neuroaxis harvested and processed for histological examination. Serial sections of the brain and spinal cord were viewed using confocal microscopy, subsequent location maps were determined. RESULTS: The corticospinal tract of pigs is located in the lateral white matter, demonstrating greater similarity to human anatomical structure than that of rodents. CONCLUSION: The corticospinal tract in pigs demonstrated anatomical similarity to human, suggesting that the porcine model may be an important translational pre-clinical model.

Poster 220 – Monday 5th December

THE NEURAL CELL ADHESION MOLECULE L1 REGULATES NEUROTRANSMITTER RELEASE IN CENTRAL NERVOUS SYSTEM SYNAPSES

Dr. Iryna Leshchyns'ka[1], Preston Ngo[1], Dr. Vladimir Sytnyk[1]

[1]University Of New South Wales

Neurotransmission plays a key role in all functions of the nervous system. Neurotransmitters in neurons are stored in specialised organelles called synaptic vesicles. Synaptic vesicles fuse with the neuronal surface membrane at synaptic contacts between neurons to release neurotransmitters via exocytosis and are then reformed in synapses by endocytosis.
The neural cell adhesion molecule L1 is highly expressed in axons of neurons in the central and peripheral nervous system. Mutations in the gene coding for L1 cause L1 syndrome, an X-linked recessive disease, which occurs in one in every 30,000 males and results in intellectual disability. The molecular mechanisms of the intellectual disability induced by L1 mutations remain however incompletely understood. We show that changes in expression of L1 in cultured hippocampal neurons affect the speed of neurotransmitter release and the speed of synaptic vesicle endocytosis. Furthermore, we demonstrate that in synapses L1 associates with proteins playing a critical role in synaptic vesicle exocytosis and endocytosis, including clathrin, which is required for clathrin-dependent endocytosis of synaptic vesicles. We also show that L1 regulates the mobility of the clathrin pits at the cell surface and formation of the clathrin-coated synaptic vesicles.
Altogether, our results indicate that L1 plays a role in regulation of the neurotransmitter release in the central nervous system. They also suggest that intellectual disability associated with abnormal function of L1 caused by L1 mutations is linked to abnormalities in neurotransmission in the brain.

Poster 221 – Tuesday 6th December

ROLE OF TDP43 IN OLIGODENDROCYTES DEVELOPMENT AND ITS IMPLICATION IN THE DISEASE PROGRESSION IN ALS AND FTD

Dr Jacqueline Y K Leung[1], Mr Samuel T Dwyer[1], Miss Rachel Atkinson[1], Prof James C Vickers[1], Dr Anna E King[1]

[1]*Wicking Dementia Research And Education Centre, University of Tasmania*

The TAR-DNA binding protein-43 (TDP-43) is one of the common pathogenic proteins involved in both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The effect of altered TDP-43 expression or aggregation in neurons has been well studied. However, TDP-43 protein aggregates are also present in oligodendrocytes from patient's post-mortem tissue. The effect of altered TDP-43 expression or its aggregation in oligodendrocytes is currently not known. The main aim of this study is to characterized the role of TDP-43 in oligodendrocyte development from their precursor cells and their ability to myelinate. Primary oligodendrocyte precursor cell cultures were prepared from postnatal day 2 rats. Lentivirus transduction of GFP-tagged human TDP-43 was used to induce TDP-43 overexpression in cultured cells. The cells were grown in media to induce the formation of mature oligodendrocytes. Images were acquired from both live and immunocytochemically labelled cultures; To analyse cell morphology, tracing of the oligodendrocyte processes was performed using Image-J to quantitate the branching complexity, an indication of cellular maturity. At 4 days post transduction, there was a significantly ($p<0.05$) higher number of branches (indicating a more complex morphology) in the transduced oligodendrocytes compare to un-transduced oligodendrocytes (n=10). These result suggest that TDP-43 overexpression accelerates the development of oligodendrocytes *in vitro*. This result supports our hypothesis that TDP-43 has an active role in oligodendrocytes development, however, further study is required to confirm its involvement in the myelination process.

Poster 222 – Monday 5th December

INSULA CORTICAL RESPONSES TO GUT STIMULATION IN SLEEP AND WAKEFULNESS

Dr Ekaterina Levichkina[1,2], Dr Ivan Pigarev[1]

[1]*Institute for Information Transmission Problems RAS,* [2]*University Of Melbourne*

INTRODUCTION: Insula is known to be involved in visceral control. Its lesions lead to both metabolic and sleep disorders. However it is unknown whether the information flow from internal organs gets reduced during slow-wave sleep, similar to information flow from external sensory modalities.
METHODS: We recorded neuronal and local field potential (LFP) responses to electrical microstimulation of intestinal wall (0.5 ms pulses, 200Hz, 150μA, 20ms trains, ISI 1 min) from/near 147 insular cells in 2 cats during sleep and wakefulness. Stimulation was titrated to the level that did not disturb animals in wakefulness. The absence of sleep disturbance after stimulation was checked by comparing EEG spectra (Wilcoxon test, $p<0.05$). Cell response was considered significant if the spike rate differed from prestimulus level by 2 SD for at least 40 ms and within this period exceeded 3 SD. The presence of evoked response in LFP was estimated using Residual Orthogonality Test.
RESULTS: From 147 cells studied, 61 cells responded to intestinal stimulation. 39 of them responded exclusively in sleep, 11 only in wakefulness and 3 cells had the same responses in both conditions. Eight cells responded excitatory in one condition and inhibitory in another. The number of responsive cells is significantly higher in sleep (Exact Fisher Test $p<0.001$). Significant evoked LFP responses were found in 15 recording sites in sleep and in 6 in wakefulness.
CONCLUSION: Afferent visceral input to insula is not attenuated but strengthened in sleep, which is consistent with the visceral theory of sleep.

Poster 223 – Tuesday 6th December

AMYLOID BETA INCREASES TAU TRANSLATION FYN DEPENDENTLY

Dr Chuanzhou Li[1], Professor Jürgen Götz[1]

[1]*Clem Jones Centre For Ageing Dementia Research, Queensland Brain Institute, The University Of Queensland*

The two hallmark lesions of Alzheimer's disease (AD) are aggregates formed by the peptide amyloid-β (Aβ) and the microtubule-associated protein Tau. We and others have shown recently that dendritic Tau mediates Aβ toxicity via the kinase Fyn in a Tau-dependent manner. This is supported by the finding that Fyn is targeted to dendritic spines by Tau, a process that is facilitated by the interaction of Tau and Fyn. Here we found that oligomeric Aβ (Aβo) induced endogenous Tau expression in cultured primary neurons, which can be blocked by an inhibition of protein translation. Using different assay, we visualized an induction of *de novo* synthesized Tau protein by Aβo which also activated the kinase Fyn in neurons. In both HEK293T cells and primary neurons, Fyn is capable of increasing exogenous and endogenous Tau expression, respectively. However, in Fyn knock-out neurons Aβo failed to stimulate Tau overexpression. Our findings suggest that Fyn has a crucial role in mediating Aβ-induced Tau pathology, shedding new light on the relationship between the amyloid plaque and the Tau pathology in AD.

Poster 224 – Monday 5th December

PROBDNF SUPPRESSES POSTNATAL NEUROGENESIS OF MICE

Miss Jia-yi Li[1]

[1]*University Of South Australia*

Postnatal neurogenesis has been shown to serve as an important sensor for different neurological disease states and is potential to provide methods of endogenous repair within the central nervous system (CNS). Despite immense interest in the therapeutic function of postnatal neurogenesis, molecular mechanism underlying this process is not fully defined. Mounting evidence indicates that brain-derived neurotrophic factor (BDNF) exerts proliferative effects on neural precursors in vivo. In this study we found the precursor of BDNF (proBDNF) regulated neurogenesis of postnatal mice. In postnatal day 4 (P4) mice, 24 h after SVZ injection of proBDNF (2□g/5□L) and anti-proBDNF antibody (25□g/□L), followed by intraperitoneal injection (IP) of BrdU (5-bromo-2'-deoxyuridine) increased proliferation in the dentate gyrus (DG) was detected by immunohistochemistry. proBDNF inhibited proliferation of neuronal cells in P5 mice reflecting the decreased neonatal neurogenesis. To further identify the neurogenesis being regulated in older mice, we performed sequential experiments in P18 mice. We found proBDNF decreased the number of newborn cells in the DG ($P<0.01$) as well and by blocking the endogenous proBDNF proliferation was increased four-fold compared with control. Together, by acute and chronic intracerebroventricular injections of proBDNF and anti-proBDNF antibody the postnatal neurogenesis of mice was inhibited and improved respectively. Our results define a critical biological function of proBDNF in neonatal neurogenesis suggesting that proBDNF regulates ongoing neurogenesis via an endocrine-like pathway coordinating proliferation, potentially providing new approaches for damaged brain recovery during neuronal development.

Poster 225 – Tuesday 6th December

THE ANTI-NEUROINFLAMMATORY EFFECT OF APIGENIN: A PILOT STUDY ON THE GFAP-IL6 NEUROINFLAMMATION MODEL

Dr Huazheng Liang[1], Ms Sandra Sonego[1], Mr Christopher Millington[1], Dr Erika Gyengesi[1], Dr Alejandra Rangel[1], Mr Paul Yoon[1], Dr Garry Niedermayer[1], A/Prof Tim Karl[1], Prof Gerald Muench[1]

[1]Western Sydney University

Background: Chronic neuroinflammation (activation of microglias and astrocytes) is a prominent pathological process in many neurodegenerative diseases, including Alzheimer's disease (AD). Consequently, it has been suggested that drugs targeting neuroinflammatory processes such as cytokine-suppressive anti-inflammatory drugs might yield clinically useful disease-modifying effect for these neurodegenerative disorders. The present study aimed to investigate the therapeutic effect of apigenin on neuroinflammation in the glial fibrillary acidic protein-interleukin 6 (GFAP-IL6) mouse in which IL6 is expressed in astrocytes using both behavioral tests and histology.
Methods: Open filed, plus maze, and Barnes maze tests were used to study the behavioral changes of GFAP-IL6 mice after 3 months treatment with apigenin. Immunohistochemical staining against Iba-1 and stereological counting were used to test the change of microglia number.
Results: Apigenin improved anxiety related behaviors and working memory after 3 months treatment on GFAP-IL6 mice. Histological staining showed that apigenin decreased the number of microglia in the cerebellum by ~30% and ~25% in the hippocampus of GFAP-IL6 mice.
Conclusion: Apigenin is a potent anti-inflammatory drug and can be potentially used for neurodegenerative diseases such as AD

Poster 226 – Monday 5th December

PERTURBATIONS IN INSULIN SIGNALLING IN ALZHEIMER'S DISEASE (AD) AND ITS CONTRIBUTION TO AD PATHOGENESIS.

Ms Julia Lim[1], Dr Boris Guennewig[2], Prof Jillian Kril[1], Dr Greg Sutherland[1]

[1]Charles Perkins Centre, The University Of Sydney, [2]Garven Institute of Medical Research, The University of New South Wales

Alzheimer's disease (AD) is a neurodegenerative disorder histopathologically characterised by the presence of amyloid beta plaques and tangles, with the latter being made up of hyperphosphorylated forms of the protein tau. AD and diabetes are two common diseases that have reached epidemic proportions in Australia. Epidemiological studies suggest that type 2 diabetes (T2D) poses a 2-3 fold greater risk of AD. Insulin resistance is one of the major hallmarks of T2D and results in a paradoxical decrease in insulin signalling in the brain. This decrease is predicted to, via the PI3K/Akt pathway, lead to an increased activation of glycogen synthase 3 beta (GSK3β), a major tau kinase. By utilizing belatedly affected regions of post-mortem AD brain as an AD model, we explored three regions: superior temporal gyrus (STG), precuneus and occipital lobe of 20 AD cases and 20 age-, gender- and APOE ε4 genotype-matched controls. No changes in key signalling molecules AKT and GSK3 were observed to support aberrant PI3K/AKT signalling in AD. Interestingly PP2A, a major tau phosphatase was decreased in AD brains as opposed to controls. RNA-Seq was also performed on control and AD mildly affected occipital lobe samples. Differentially expressed genes included insulin-like growth factor receptor and somatostatin levels that were up- and down-regulated in AD, respectively.

Poster 227 – Tuesday 6th December

EXTINCTION AND LATENT INHIBITION INVOLVE A SIMILAR FORM OF INHIBITORY LEARNING THAT IS STORED AND RETRIEVED IN THE INFRALIMBIC CORTEX

Dr. Nura Lingawi[1], Prof Frederick Westbrook[1], Dr Vincent Laurent[1]

[1]University Of New South Wales

Extinction and latent inhibition each refer to a reduction in conditioned responding: the former occurs when pairings of a conditioned stimulus (CS) and an unconditioned stimulus (US) are followed by repeated presentations of the CS alone; the latter occurs when CS alone presentations precede its pairings with the US. The present experiments used fear conditioning to test the hypothesis that both phenomena involve a similar form of inhibitory learning that recruit common neuronal substrates. We found that the inhibitory memory established by fear extinction is reactivated in the infralimbic cortex (IL) during additional extinction. Remarkably, this reactivation also occurs when the initial inhibitory memory is established in latent inhibition. In both cases, the inhibitory memory was strengthened by pharmacological stimulation of the IL. Moreover, NMDA receptor blockade in the IL disrupted the weakening in conditioned responding produced by either latent inhibition or extinction. These findings therefore indicate that latent inhibition and extinction produce a similar inhibitory memory that is retrieved in the IL. They also demonstrate that the IL plays a wide role in fear regulation by promoting the expression of inhibitory memories generated by CS alone exposure either before or after this CS has been rendered dangerous.

Poster 228 – Monday 5th December

NEUREGULIN 1 TYPE III "KNOCKOUT" MICE SHOW HEIGHTENED SENSITIVITY TO THE NEUROBEHAVIOURAL EFFECTS OF CANNABIS

Mr David Lloyd[1,2], Mr Juan Olaya[1,2], Mrs Cyndi Shannon-Weickert[1,2], Mr Tim Karl[1,2]
[1]Neuroscience Research Institute Australia, [2]Schizophrenia Research Institute

Objective
Experimental studies have found that mice possessing a transgene that affects all isoforms of the schizophrenia candidate gene, Neuregulin 1, have a modified neurobehavioural response to cannabis constituents. The current mouse study investigates whether the *Nrg1* type III isoforms are responsible for this phenomenon.
Method
Adult, male heterozygous knockout mice for *Nrg1 type III* (*Nrg1 III* KO) and wild type-like control littermates (WT) were treated via i.p. injection with either vehicle (i.e. saline) or 3 or 10 mg/kg THC, the main psychoactive component of cannabis, then tested for locomotion, anxiety and sensorimotor gating 30 min later.
Key findings
In open field, both 3mg/kg and 10mg/kg THC led to a significant decrease in locomotion and a significant increase in distance ratio (a proxy for anxiety). However the effects of THC were significantly stronger in *Nrg1 III* KO mice vs WT for 3mg/kg (distance travelled: $F(2, 70) = 3.46$, $p = 0.02$; distance ratio ($F(2, 70) = 4.75$, $p = 0.001$), but not for 10mg/kg. We did not find any *Nrg1 III* KO x THC interactions for PPI.
Conclusions
The current study suggests that *Nrg1 III* KO mice may be more susceptible to the neurobehavioural effects of THC on locomotion and anxiety-related behaviours, but not PPI. Future investigations that focus on the molecular effects of *Nrg1 III* KO vs other Nrg1 transgenes and the brain response to THC will be able to shed light on why this might be the case

Poster 229 – Tuesday 6th December

THE DEVELOPMENT AND GENERATION OF ORIENTATION SELECTIVITY IN THE VISUAL SYSTEM: A COMPUTATIONAL STUDY

Mr Errol Kerry John Lloyd[1], Dr Catherine E Davey[3], Prof Anthony N Burkitt[3], Prof Trichur Raman Vidyasagar[1,2]

[1]Department of Optometry and Vision Sciences, The University of Melbourne, [2]Melbourne Neuroscience Institute, The University of Melbourne, [3]Department of Electrical & Electronic Engineering, The University of Melbourne

INTRODUCTION: Two aspects of the lateral geniculate nucleus (LGN) warrant further attention concerning it's role in cortical visual function. First, projections from retina to LGN are divergent, whilst LGN to primary visual cortex (V1) projections are both convergent and divergent. Thus, multiple LGN cells share similar receptive fields, common retinal input and are correlated in their firing. Conversely, V1 cells receive input from many LGN cells, which share similar receptive fields and retinal inputs (Alonso et al., 2006). Second, both retinal and LGN cells exhibit biases to orientation (Vidyasagar & Eysel, 2016). Together, these facts suggest that there may be an unbroken functional stream linking the orientation biases of the retina and the well-known orientation selectivity of V1, which may explain both the development and generation of V1 orientation selectivity.

METHODS: The retina-LGN-V1 pathway was modelled by three layers of neurones. LGN to V1 projections were initially random and diffuse and allowed to evolve according to Hebbian plasticity whilst being driven by noisy spontaneous firing in the first layer. With the retinal layer neurones possessing orientation-biased receptive fields, V1 neurones were functionally assessed with sine-wave gratings

RESULTS: The inputs to V1 neurones (~70%) developed from being spatially diffuse and unselective to more confined and orientation selective, with the change in circular variance for all V1 'neurones' being significantly negative (ie sharper orientation selectivity) (Wilcoxon, $Z = -15.25$, $p<0.001$).

CONCLUSION: The retinal orientation biases and retinogeniculate divergence can lead to the unsupervised development of cortical orientation selectivity.

Poster 230 – Monday 5th December

ORIENTATION SELECTIVITY IN THE SUPERIOR COLLICULUS OF TREE SHREWS

Ms Yamni S Mohan[1], Dr Sivaram Viswanathan[1], **Mr Errol Kerry John Lloyd[1]**, Dr Jaikishan Jayakumar[1,2], Prof Trichur Raman Vidyasagar[1,3]

[1]Department of Optometry and Vision Sciences, The University of Melbourne, [2]Centre for Computational Brain Research, Indian Institute of Technology, [3]Melbourne Neuroscience Institute, The University of Melbourne

INTRODUCTION: The superior colliculus (SC) of the tree shrew receives direct inputs from the retina in parallel to the retinogeniculate projection. An early study reported that only a small proportion (approx. 12%) of SC neurons had elongated receptive fields and were biased for stimulus orientation (Albano et al., 1978). We revisited this claim, since moderate degrees of orientation sensitivity are now described as very common in the retinogeniculate projection in a number of species.

METHODS: We recorded from the SC in three anaesthetised tree shrews. A neuron was deemed orientation selective if the circular variance (CV) of the response to relatively thin, oriented bars was smaller than or equal to 0.90. We also measured orientation tuning using moving gratings of different spatial frequencies.

RESULTS: 17 of 18 recorded neurons demonstrated significant orientation biases (Median CV= 0.84, One-sample Wilcoxon signed rank test, z= -3.3752, p<0.001 for null hypothesis- median CV= 0.9). When using gratings, these neurons showed significant orientation biases at higher spatial frequencies, which explains why these were missed in earlier studies that did not use either fine bars or gratings.

CONCLUSION: The ubiquitous finding of orientation biases in the tree shrew SC, similar to the retinogeniculocortical pathway in all species studied so far, gives further support to the notion that orientation selectivity is already present in the retina, getting further elaborated in different parts of the brain rather than generated first in the primary visual cortex.

Supported by ARC Centre of Excellence in Integrative Brain Function.

Poster 231 – Tuesday 6th December

NEUROPATHOLOGY AND NOVEL MOUSE MODELS OF RAB39B-MEDIATED PARKINSON'S DISEASE

Gabrielle Wilson[1,2], Yujing Gao[1,2], Mirella Dottori[3], Sarah Stephenson[1,2], Catriona McLean[4], Paul Thomas[5], **Paul Lockhart[1,2]**

[1]Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, [2]Department of Paediatrics, University of Melbourne, [3]Centre for Neural Engineering, University of Melbourne, [4]Alfred Hospital, Anatomical Pathology, [5]School of Biological Sciences, The University of Adelaide

We previously demonstrated that loss-of-function mutations in *RAB39B* cause early-onset PD and subsequent reports have demonstrated clinically typical, levodopa responsive PD with early and late onset due to mutation of *RAB39B*. We have performed post-mortem neuropathological studies in two individuals with RAB39B-mediated disease due to complete *RAB39B* deletion or a pathogenicT168K point mutation. In both cases the pathological features included loss of dopaminergic neurons in the substantia nigra and classic lewy pathology. Additional findings included tau immunoreactivity, axonal spheroids and iron accumulation. CRISPR/CAS9 editing was used to generate mouse models of both the T168K mutation and *Rab39B* KO. Western blot analyses demonstrated significantly reduced or absent Rab39b in the T168K and KO brains respectively, confirming the mice accurately model the human disease mechanism. Ongoing behavioural analysis of a cohort of KO mice demonstrated a movement phenotype consistent with dysregulation of nigrostriatal signalling at ~12 months of age. Sustained hindlimb clasping with trunk flexion was observed and the KO mice performed significantly worse than littermate controls on the balance beam, slipping ~4 times as often (1.9±0.7, n=10 Vs 7.6±2.0, n=10; P=0.009). Our results suggest these unique models accurately recapitulate the human disease mechanism and will be useful to dissect disease pathogenesis and test therapeutic strategies.

Poster 232 – Monday 5th December

LOSS OF AMPK IN NPY/AGRP NEURONS ALTERS FEEDING IN RESPONSE TO GLUCOSE AVAILABILITY IN MICE.

Dr Sarah Lockie[1], Dr Romana Stark[1], Ms Clare McAuley[1], Dr Zane Andrews[1]

[1]Monash University

Metabolic feedback from the periphery to the brain comes from a dynamic physiologic fluctuation of nutrients and hormones, including glucose and fatty acids, ghrelin, leptin and insulin. With this view, we hypothesised that glucose availability may alter ghrelin-induced feeding behaviour. To modulate endogenous glucose metabolism we injected mice (ip) with glucose (2.25g/kg) or 2-deoxyglucose (2DG; 250mg/kg) to simulate hyper- and hypoglycaemia respectively. 30 minutes later we injected ghrelin either ip or ICV. Food intake was measured 2 hours later. In all experiments, glucose suppressed ghrelin-induced feeding and 2-DG potentiated ghrelin-induced feeding. The canonical target of ghrelin in the brain is the NPY/AgRP neurons of the arcuate nucleus of the hypothalamus. As AMPK is a key metabolic sensing molecule, and a downstream modulator of ghrelin signalling, we virally knocked down AMPK in NPY/AgRP neurons to interrogate its role in these feeding effects. AMPK knockdown did not affect sensitivity to ghrelin alone, but did significantly decrease sensitivity the feeding response to 2DG. When ghrelin sensitivity was measured following either 2-DG or glucose administration in AMPK NPY/AgRP KD mice, there was no difference to the overall shape of the 2-DG ghrelin effect, with 2-DG and ghrelin still additively increasing food intake. Interestingly, glucose pretreatment was no longer able to supress ghrelin-induced feeding in AMPK NPY/AgRP KD mice, an effect which was

robust in WT mice. These data demonstrate that AMPK in NPY/AgRP neurons is critical for modulating feeding response to 2-DG-induced glucopenia and for the inhibitory effect of glucose on ghrelin-induced feeding.

Poster 233 – Tuesday 6th December

ASSESSING APP96-110 AS A NOVEL NEUROPROTECTIVE AGENT IN TRAUMATIC SPINAL CORD INJURY

Ms Sarah Lovett[1], Emeritus Professor Alan Harvey[1], Associate Professor Giles Plant[2], Associate Professor Corinna van den Heuvel[3], Associate Professor Stuart Hodgetts[1]

[1]Spinal Cord Repair Laboratory, School of Anatomy, Physiology and Human Biology, The University Of Western Australia, [2]Department of Neurosurgery, Stanford Partnership for Spinal Cord Injury and Repair, Stanford University School of Medicine, [3]School of Medical Sciences, The University of Adelaide

Spinal cord injury (SCI) results in disruption of axons and extensive cell death at the immediate injury site. Secondary degenerative events contribute to the widespread and ongoing loss of surrounding spared tissue. Early interventions that protect the spinal cord from secondary degeneration could increase tissue sparing, preserve functional connections and improve repair at the injury site. A heparin binding region at residues 96-110 of the amyloid precursor protein (APP) has marked neuroprotective effects following traumatic brain injury, and may have similar effects following SCI. We investigated the neuroprotective effect of the APP96-110 peptide, alone and in combination with human mesenchymal precursor cell (hMPC) transplantation, which significantly improves functional and morphological outcomes following SCI. Rats received a moderate contusive SCI and were treated with intravenous APP96-110 (active) or mAPP96-110 (inactive mutant) peptide at 30mins post-injury. A cohort of animals received intralesional viable/non-viable (nv) hMPC transplantation at 1wk post-injury (wpi). There were no significant differences in functional outcomes assessed with open-field, Ladderwalk or RatWalk between treatment groups at 8wpi. Transplanted hMPCs survived to 8wpi in both viable hMPC transplant groups while no nv-hMPCs survived to 8wpi. There was a significant reduction in cyst size with combined APP96-110+hMPC treatment (6.5%) compared to SCI only (20%), which was not seen with other treatments. This preliminary study shows that the APP96-110 peptide may have neuroprotective effects following SCI. We are investigating whether increased peptide concentration, repeated injection or alternative routes of administration, alone and in combinatorial approaches, may give more significant improvements following SCI.

Poster 234 – Monday 5th December

VITAMIN D TREATMENT DURING PREGNANCY BLOCKS MATERNAL IMMUNE ACTIVATION INDUCED BEHAVIORAL PHENOTYPES IN OFFSPRING VIA ITS NEUROPROTECTIVE EFFECTS ON THE ONTOGENY OF DOPAMINE NEURONS

Dr Wei Luan[1], Dr Stephanie Vulliermot[2], Dr Urs Meyer[2], Ms Suzanne Adele Alexander[3], Dr Darryl Eyles[1,3]

[1]Queensland Brain Institute, the University Of Queensland, [2]Swiss Federal Institute of Technology (ETH) Zurich, [3]Queensland Centre for Mental Health Research

Exposure to infection during critical prenatal developmental periods is a well-validated environmental risk factor for developmental neuropsychiatric disorders such as schizophrenia. Considering the immunomodulatory and neuroprotective functions of vitamin D, we investigated whether treatment with calcitriol, the active form of vitamin D, could prevent poly (I:C)-induced behavioral disturbances. By co-delivering poly (I:C) with calcitriol to pregnant mice at gestation day 9, we examined (1) inflammatory markers in dam and fetus post-treatment, (2) the amphetamine-induced locomotor activity of offspring at postnatal day 35 and 70, (3) and the embryonic mesencephalic dopamine neurodevelopment. We show treatment with calcitriol completely blocked the amphetamine-induced hyperlocomotion induced by poly (I:C) in offspring at both adolescent and adult stages. This was clearly not due to the putative anti-inflammatory properties of vitamin D as inflammatory cytokine production was unaffected in either dams or fetuses. Quantitative immunochemical analysis revealed that treatment with calcitriol increased the nuclear density of the essential specification factor for dopamine neurons, Nurr1, in post-mitotic dopamine neurons in poly (I:C)-exposed embryos when compared to the saline-treated. However, the treatment with calcitriol did not block the reduction of dopamine neuronal number induced by poly (I:C). This strongly suggests vitamin D protects the development of mesencephalic dopaminergic system from the adverse effects of the poly (I:C) exposure independent of its immunomodulatory role. This work raises the possibility for future preventative maternal supplementation strategies to prevent developmental neuropsychiatric disorders using dietary vitamin D.

Poster 235 – Tuesday 6th December

OPTOGENETIC STIMULATION OF THE MIDBRAIN COLLICULI EVOKES CARDIORESPIRATORY RESPONSES IN THE BEHAVING RAT

Ms Erin Lynch[1], Dr Bowen Dempsey[1], Dr Peter Burke[2], Prof Roger Dampney[3], A/Prof Ann Goodchild[1], Dr Simon McMullan[1]

[1]Macquarie University, [2]Neuroscience Research Australia, [3]University of Sydney

The superior and inferior colliculi play key roles in the immediate processing of threatening sensory stimuli and generate rapid behavioural responses that are critical for survival. In addition to its well-documented role in sensory processing, we have previously found that disinhibition of the colliculi in anaesthetised animals unmasks coordinated respiratory, sympathetic and somatomotor outputs that are independent of processing in higher centres. In the present study we investigate whether such effects are observable in the behaving

animal, and examine the evidence for a previously unreported direct projection from the colliculi to cardiorespiratory control centres in the brainstem.
We used an AAV vector to express Channelrhodopsin2 (ChR2) in the colliculi and instrumented animals to record EEG and allow photoactivation of ChR2-expressing neurons via a chronically implanted fibre optic. Respiratory rate was measured by whole animal plethysmography. Colliculi photoactivation in freely behaving rats evoked distinct and reproducible behavioural response characterised by freezing, orienting and circling toward the contralateral side, and a concurrent increase in ventilation rate (45 ± 12%, $P<0.01$, N=7). Colliculi photoactivation also desynchronized EEG, driving immediate predominance of the theta band. Smaller but qualitatively similar respiratory effects were accompanied by tachycardia under anaesthesia. No effects were observed in animals injected with a control vector that does not drive ChR2 expression.
We conclude that optogenetic collicular activation can drive cardiorespiratory effects that are consistent with our previous findings; ongoing experiments will determine whether these effects are mediated via activation of a collicular efferent pathway that terminates in the ventromedial medulla.

Poster 236 – Monday 5th December

INVESTIGATING THE ROLE OF DOPAMINE RECEPTORS AND PARVALBUMIN-EXPRESSING NEURONS IN EXTINCTION OF CONDITIONED FEAR

Dr Heather Madsen[1,2], Mr Alexandre Guerin[1,2], Dr Jee Kim[1,2]

[1]Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, [2]Florey Department of Neuroscience and Mental Health, University of Melbourne

Objective: Post-traumatic stress disorder affects more than 800,000 Australians. It is currently treated using exposure therapy, which can be modelled in the laboratory using Pavlovian fear conditioning and extinction. A better understanding of the neural circuitry underlying extinction is critical for developing effective therapies. Therefore, we examined the pattern of activation of neurons that express dopamine receptors 1 and 2 (D1R and D2R), and parvalbumin (PV) in mice that underwent extinction or retrieval of a fear memory.
Methods: Adult male transgenic mice expressing D1R or D2R tagged with green fluorescent protein (GFP) were fear conditioned with 6 tone-shock pairings. The following day they were randomly divided into one of four experimental groups: handled, context, retrieval or extinction. Extinction groups were exposed to 45 tone presentations, retrieval groups were exposed to 5 tone presentations and the context groups were exposed to the extinction context without any tones. 90 minutes following their assigned treatment, mice were perfused and brain tissue processed for Fos/GFP/PV immunohistochemistry.
Results: Quantification of immunoreactivity revealed that extinction led to increased D2 expression in the infralimbic cortex, and decreased D2 expression in the prelimbic cortex, as well as increased D1 neural activation in the amygdala. Fear memory retrieval resulted in increased D1 and D2 neural activation in the prelimbic cortex, and increased D1 and D2 neural activation in the infralimbic cortex.
Conclusion: Fear extinction and retrieval engage overlapping as well as distinct neural circuitry involving dopamine. These results provide important insights into improving extinction using dopamine in the future.

Poster 237 – Tuesday 6th December

LKB1/AMPK/M-TOR RESPONSE TO ISCHEMIA IN HIGH METABOLIC RATE TISSUES: A COMPARISON BETWEEN THE BRAIN, HEART, KIDNEY AND LIVER INTRACELLULAR METABOLIC AXIS ACTIVATION IN ISCHEMIC SITUATION

Dr. Shohreh Majd[1], Associate Professor John Power[1], Professor Simon Koblar[2], Professor Hugh Grantham[1]

[1]Flinders University, [2]Adelaide University

The cellular energy depletion due to ischaemia is one of the most stressful situations for the cells, in particularly for those with a high metabolic rate. To protect the cells against the consequence of a metabolic disturbed situation, the energy sensing Liver Kinase B1 (LKB1)/Adenosine Monophosphate Kinase Protein Kinase (AMPK) pathway regulates the cellular balance of energy. Here we studied the activation of LKB1/AMPK axis along with their down-steam regulatory kinase, Mammalian target of rapamycin (mTOR) in response to a time-dependent ischaemic situation (15 sec to 8 min) in the brain, heart, kidney and liver using a rat model of reversible cardiac arrest (CA). Our results demonstrated that LKB1 phosphorylation decreased in the brain after 30 sec ischaemia, while it increased after 4 min in the heart and kidney, and remained unchanged in the liver. AMPK activity increased in the brain after 8 min following a significant decrease in the shorter periods of ischaemia, decreased in up to 1 min ischaemia in the kidney and showed an increase in the heart after 8 min of ischaemia. m-TOR phosphorylation didn't show any alteration in the heart, kidney and liver, while it increased after 4 and 8 min of ischaemia. We concluded that LKB1/AMPK/m-TOR axis acts in different ways in response to the same duration of ischemia, such as a less phosphorylation level of the first sensors in a tissue with less glucose resources (brain) as an early response, but the same or the higher phosphorylation levels for the tissues with some glucose resources such as the liver, kidney or the heart.

Poster 238 – Monday 5th December

ADENOSINE MONOPHOSPHATE PROTEIN KINASE (AMPK) TRIGGERS TAU HYPERPHOSPHORYLATION AT SER262 IN RESPONSE TO ALTERATION IN NEURONAL BLOOD SUPPLY: AN IMPLICATION FOR METABOLIC BASED HYPOTHESIS FOR ALZHEIMER'S DISEASE

Dr. Shohreh Majd[1], Associate Professor John Power[1], Professor Simon Koblar[2], Professor Hugh Grantham[1]

[1]Flinders University, [2]University of Adelaide

Increased tau phosphorylation (p-tau) is a causative agent of Alzheimer's disease (AD), and a consequence of brain hypoxia. Demonstrating the effect of residues-specific tau hyperphosphorylation on cognition, leads to understanding of the pathology of memory impairment. Here we studied the cognition behaviour in a rat model of reversible cardiac arrest (CA). The activity of the main cellular metabolic axis, Liver Kinase B1 (LKB1)/Adenosine Monophosphate Kinase Protein Kinase (AMPK), along with the activity of Glycogen Synthase Kinase (GSK-3β), Protein Phosphatase 2A (PP2A) and tau-specific phosphorylation at AMPK-sensitive epitope and GSK-3β- sensitive epitope were assessed in both rats and human AD brains. Our results demonstrated that LKB1/AMPK activity increased 4 weeks after CA as did AMPK specific p-tau (Ser^{262}), however generating cognition deficits required the further involvement of GSK-3β, and PP2A, as the unchanged activity of them, along with the constant level of p-tau (Ser^{202}/Thr^{205}), was not associated with memory impairment. In AD brains, both AMPK and GSK-3β were activated, while PP2A showed less activity. We concluded that to create cognitive deficits, hyperphosphorylation of tau in more than one specific site and the contribution of more than one tau kinase is required.

Poster 239 – Tuesday 6th December

CHARACTERISING PERIPHERAL AND CENTRAL SENSORY NERVE FIBRE CHANGES ASSOCIATED WITH CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Mr Preet Makker[1], Dr Chamini Perera[1], Mr Samuel Duffy[1], Dr Justin Lees[1], Mr Ryan Tonkin[1], Dr Gila Moalem-Taylor[1]

[1]Neuropathic Pain Research Group, Translational Neuroscience Facility, University of New South Wales

Chemotherapy-induced peripheral neuropathy (CIPN) and associated neuropathic pain can severely affect the quality of life of cancer patients during and after treatment with drugs, such as paclitaxel (an anti-tubulin chemotherapeutic). Current understanding of chemotherapy-induced changes in the nervous system is limited. Here, we used a mouse model of paclitaxel-induced peripheral neuropathy to characterise changes in sensory nerve fibres in the peripheral and central nervous system. We found an increase in ATF-3 expression in dorsal root ganglion sensory neurons of paclitaxel-treated mice compared to saline controls. The expression of ATF-3 was found to be co-localised in IB4+ non-peptidergic C-fibre sensory neurons and NF200+ myelinated sensory neurons. However, proportions of NF200+, IB4+ and CGRP+ neurons remained unchanged. In the spinal cord, expression of IB4 nerve terminals in lamina 1 and 2 region of the dorsal horn was reduced in paclitaxel-treated mice, with no changes in expression of CGRP nerve terminals. In summary, we observed damage to myelinated and unmyelinated sensory neurons and a reduction in non-peptidergic C-fibre nerve terminals in spinal cord lamina 1 and 2 following paclitaxel treatment. Our results highlight underlying pathological changes in paclitaxel-induced peripheral neuropathy that may give rise to neuropathic pain.

Poster 240 – Monday 5th December

ELUCIDATING THE ROLE OF METALS IN THE DOWN SYNDROME BRAIN

Ms Nakisa Malakooti[1], Dr Melanie Pritchard[2], A/P Ronald C Kim[3], Prof Ira T Lott[3], Ms Irene Volitakis[1], Dr Blaine R Roberts[1], Prof Catriona A McLean[1], A/P David I Finkelstein[1], A/P Paul A Adlard[1]

[1]The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, [2]Department of Biochemistry and Molecular Biology, Monash University, [3]University of California

Introduction: Down syndrome (DS) is the most common intellectual disability, with an incidence of 1 in 700 births and is caused by whole or partial trisomy of chromosome 21. All people with DS develop Alzheimer's disease (AD)-like neuropathology by the age of 40. One of the characteristics of AD is dyshomeostasis of metals in the brain.
Objective: Since one of the characteristics of AD is dyshomeostasis of metals in the brain, we tested whether metal homeostasis was also altered in the DS brain.
Method: We measured metal levels in the hippocampus, prefrontal and temporal cortices in nineteen post mortem DS brains of individuals who had developed AD (55.9 ± 7.2 years of age) and seven control brains (54.2± 3.07 years of age) by inductively coupled plasma mass spectrometry (ICPMS). Liquid chromatography-ICPMS (LC-ICPMS) was also conducted to characterize metalloproteome in the DS brain.
Result: Iron levels were significantly higher and calcium levels were significantly lower in the hippocampus, prefrontal and temporal cortices in DS. Zinc levels were significantly lower in the DS temporal cortex. The distribution of metals in the DS brain were different to the control.
Conclusion: These data suggest that metals are dysregulated in DS. However, the underlying cellular mechanisms of this failure in metal ion homeostasis are yet to be explored.

Poster 241 – Tuesday 6th December

SYSTEMIC ADMINISTRATION OF A CONNEXIN43 MIMETIC PEPTIDE IN RATS IMPROVES FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

Mr Yilin Mao[1], Mr Ryan Tonkin[2], Ms Tara Nguyen[1], Dr Simon O'Carroll[3], Prof Louise Nicholson[3], Prof Colin Green[4], Dr Gila Moalem-Taylor[2], Dr Catherine Gorrie[1]

[1]Neural Injury Research Unit, School of Life Sciences, Faculty of Science, University of Technology Sydney, [2]Neuropathic Pain Research Group, Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, [3]Department of Anatomy and Medical Imaging and the Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, [4]Department of Ophthalmology, Faculty of Medical and Health Sciences, University of Auckland

Connexin43 is the main gap junction protein on astrocytes in the central nervous system, Blocking Connexin43 hemichannels has been shown to limit neural damage in a number of models. Peptide5, a Connexin43 mimetic peptide, has been shown to reduce secondary damage in spinal cord injury via local administration. We have investigated whether Peptide5 can also target the lesion area and protect the spinal cord when delivered systemically in a rat model of contusion injury. Rats were subjected to a mild spinal cord contusion using the MASCIS impactor and were injected intraperitoneally with Peptide5 (n=16) or a scrambled peptide (n=16) immediately and at 2 and 4 hours post-injury. Rats were tested for locomotor recovery and pain hypersensitivity and then euthanised at 6 weeks post-injury. When compared to controls, Peptide5 treated rats showed significant improvement in hindlimb locomotor function between 3 and 6 weeks post-injury and reductions in at-level mechanical allodynia from week 1 post-injury. At 6 weeks post-injury, lesion size, the astrocytic (GFAP) and the activated macrophage and/or microglial (ED1/Iba1) responses were all decreased in the Peptide5 treated animals compared to those treated with the scrambled peptide. In addition, neuronal cell (NeuN) numbers were higher at 6 weeks post-injury in the Peptide5 animals in comparison with the scrambled peptide treated rats. These findings provide strong *in vivo* data supporting the benefits of systemic delivery of Peptide5, as a feasible approach for acute spinal cord injury, in reducing secondary injury and improving functional recovery

Poster 242 – Monday 5th December

ADAR1 DUAL FUNCTION AS RNA EDITING AND POTENTIAL DNA BINDING ENZYME IN THE ACTIVITY-DEPENDENT REGULATION OF ADAPTIVE BEHAVIOUR IN THE MOUSE

Mr. Paul Marshall[1]
[1]University Of California Irvine

RNA editing enzymes have been known for some time to affect the qualitative and quantitative nature of RNA products. RNA editing has also been correlatively linked to the mediation of behaviour of organisms ranging from flies to humans, with the highest levels of these enzymes in humans. Mechanistically, this has been suggested to occur by their ability to bind to RNA and participate in deamination of: cytosine in the case of APOBECs', or adenosine in the case of ADARs'. However, two caveats arise in the proposed relationship. One is that not all variants of these enzymes appear to operate solely by this mechanism; they can also bind to DNA. And two, that much of the mechanistic work for this latter point has been divorced from a behaviourlly relevant context. Therefore, in order to assess the function of these domains in the context of behavioural adaptation, genome-wide DNA sequencing for ADAR1 was performed both with cultured primary cortical neurons (PCN's) and 6-8 week old C57 mice subjected to fear conditioning and extinction. An shRNA was also designed against ADAR1 and transfected with PCN's and into the infralimbic cortex of the trained mice. It has been observed that ADAR1 appears to bind a number of targets on DNA. Additionally and surprisingly, it was observed that although mRNA and protein levels appeared elevated specifically to extinction behaviour, following knockdown of ADAR1 in the infralimbic cortex the expression of fear was enhanced, while fear extinction remained unaffected. It remains to be seen whether this effect is mediated primarily by ADAR1's RNA editing activity or the binding capacity to DNA.

Poster 243 – Tuesday 6th December

INACTIVATION OF PI 3-KINASE P110Δ PREVENTS COGNITIVE DECLINE AND REDUCES PLAQUE DENSITY AND NEUROINFLAMMATION IN A MURINE MODEL OF ALZHEIMER'S DISEASE

Dr. Ramon Martinez-marmol[1], Dr. Iris Wang[1], Ms. Nika Mohannak[1], Dr. Marc Ruitenberg[2], Professor Jenny Stow[3], Professor Bart Vanhaesebroeck[4], Professor Elizabeth Coulson[1], Professor Frederic Meunier[1]

[1]University Of Queensland-Queensland Brain Institute, [2]University of Queensland-School of Biomedical Sciences, [3]University of Queensland-Institute for Molecular Biosciences, [4]University College London

Alzheimer's disease (AD) is associated with the accumulation of a neurotoxic protein, amyloid-β (Aβ), which leads to nerve degeneration, neuronal death and cognitive decline. Another defining molecular event that contributes to the pathogenesis of AD is a profound inflammatory response elicited by Aβ. We have previously shown that systemic inhibition of the p110δ isoform of phosphoinositide 3-kinase (PI3Kδ) is neuroprotective in ischemic stroke by controlling tumor necrosis alpha (TNFα) production by microglia. In the current study we describe a novel role for PI3Kδ in APP trafficking within neurons. $APP_{swe}/PS1^{\Delta E9}$ (APP/PS1) familial AD model mice were crossed with mice that carry an inactive PI3Kδ allele (δ^{D910A} mice). Here we report that inactivation of PI3Kδ significantly decrease the forward trafficking of neuronal APP *in vitro* and reduces TNFα levels *in vivo* in the brains of APP/PS1 mice. Furthermore, whereas APP/PS1 mice exhibit spatial learning and memory deficits, their littermates lacking PI3Kδ activity are cognitively normal, display a minimal inflammatory response and have significantly fewer Aβ plaques. The percentage survival rate at 7 months was also higher in the latter case. These data demonstrate that inhibition of PI3Kδ has the potential to protect against AD pathology through a dual complementary mechanism, simultaneously affecting APP trafficking in neurons and TNFα production in microglia.

Poster 244 – Monday 5th December

CHARACTERIZATION OF GANGLION CELL POPULATIONS IN MARMOSET RETINA.

Miss Rania Masri[1,2], Dr. Kumiko Percival[1], Dr. Amane Koizumi[3], Prof. Paul Martin[1,2], Assoc. Prof. Ulrike Grünert[1,2]

[1]Department of Ophthalmology and Save Sight Institute, The University of Sydney, [2]Australian Research Council Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]National Institutes of Natural Sciences

Three well described types of ganglion cells in primate retina form the bulk of the ganglion cell population; they are midget, parasol, and small bistratified cells. In addition, there is a variety of low density wide-field ganglion cell types, but the exact numbers and proportions of these cells are not known. Here, we characterized retinal ganglion cells in the marmoset using particle-mediated gene-transfection. Quadrants of retinal tissue were transfected using expression plasmid for the postsynaptic density 95-green fluorescent protein. The retinas were cultured for 3 days, fixed, then processed with bipolar and amacrine cell markers in order to determine ganglion cell dendritic stratification. Ganglion cells were classified based on dendritic field size, morphology and stratification in the inner plexiform layer. In total over 500 ganglion cells were classified into at least 17 types, including midget, parasol, broad thorny, small bistratified, large bistratified, recursive bistratified, recursive monostratified, narrow thorny, smooth monostratified, large sparse, giant sparse ganglion cells, and a group that may contain several as-yet uncharacterized types. Assuming each characterized type forms a hexagonal mosaic, midget and parasol cells account for over 80% of all ganglion cells in central retina but only ~50% of cells in peripheral (>2 mm) retina. Thus, in primate, the fovea is dominated by midget and parasol cells, but outside the fovea the ganglion cell diversity is likely as great as that reported for non-primate retinas.

Poster 245 – Tuesday 6th December

mRNA EXPRESSION OF NEUROGENESIS GENES IS CHANGED OVER THE HEALTHY HUMAN LIFE SPAN – A CONSEQUENCE OF GLIAL CHANGES?

Ms Kathryn Mathews[1], Dr Katherine Allen[2], Mr Danny Boerrigter[2], Professor Cynthia Shannon Weickert[2], Associate Professor Kay Double[1]

[1]Discipline of Biomedical Science and Brain and Mind Centre, Sydney Medical School, The University Of Sydney, [2]Neuroscience Research Australia, Schizophrenia Research Institute and School of Psychiatry, The University of New South Wales

The dentate gyrus of the hippocampus is one of two neurogenic regions in the adult brain. The proliferation and differentiation of stem cells within the adult hippocampus is thought to decline throughout the healthy lifespan and has been suggested to contribute to dementia risk in older people. In this study, we investigated changes to mRNA and protein expression of neurogenesis and glial markers over the healthy human life span.
Twenty-six human post-mortem cases were utilised, aged 18-88 years old. Quantitative reverse transcriptase polymerase chain reaction was used to quantify expression of the genes Ki67 (MKI67; cellular proliferation), doublecortin (DCX; immature neurons) and glial fibrillary acidic protein (GFAP; astrocytes and stem cells) with the relative standard curve method. These quantities were normalised to the geomean of three housekeeper genes. Immunofluorescence staining was also undertaken in five cases aged 18-88 for the microglial proteins ionised calcium-binding adapter molecule 1 (Iba1) and human leukocyte antigen DR (HLA-DR).
Increased age predicted the declining expression of Ki67 (r= -0.512; p=0.009) and DCX (r= -0.600; p=0.001) and increasing expression of GFAP (r= 0.598; p=0.036). Morphological and phenotypic changes to microglia suggested an increase in pro-inflammatory glial activation with age.
These data indicate a decline in cell proliferation and neuronal differentiation with age. We hypothesise that age-related changes of the glial environment, evidenced by increases in GFAP expression and putatively to microglial cells, may contribute to this decline. These data suggest that reducing inflammation may maintain neurogenesis in the ageing human hippocampus and thus support cognitive function.

Poster 246 – Monday 5th December

THE EFFECTS OF AGEING ON SPONTANEOUS INHIBITORY AND EXCITATORY CURRENTS IN DORSAL HORN NEURONS

Mayhew JA, Callister RJ, Smith D, Graham BA

University of Newcastle, Callaghan

Altered pain states are more prevalent among the elderly, however, the basis of this observation remains unclear. The spinal dorsal horn (DH) is a key site for sensory processing, and disruption to processing in this region can cause allodynia and hyperalgesia. To assess if changes in the DH contribute to the higher incidence of pain in the elderly, we assessed inhibitory and excitatory synaptic transmission in young (3-4 months) and aged (28-32 months) mice. Spinal slices were prepared from the lumbar spinal cord for patch clamp recording. During inhibitory recordings, mixed inhibition (GABAergic and glycinergic) was assessed and the GABAergic component was then block (bicuculline). Mixed inhibitory currents from aged and young animals had similar amplitudes (-46.73 v -40.83pA, Aged v Young), frequencies (0.59 v 0.71Hz), and rise times (2.6 v 2.3ms); but decay times were slower in the aged recordings (30.1 v 21.3ms, p=0.02) suggesting a greater GABAergic component. This was supported by the effect of bicuculline, which reduced frequency (0.62 v 0.31Hz, p=0.0005) and decay times (32.1 v 22.9ms, p=0.031) in aged recordings only. Excitatory currents recorded from aged animals had reduced amplitudes (-15.3 v -25.0pA p=0.001), but similar frequencies (4.0 v 2.0 Hz), rise (1.24 v 1.23ms) and decay times (5.0 v 4.8ms). Thus, both excitatory and inhibitory synaptic transmission is altered in the aged DH. This would be predicted to alter the baseline activity of these circuits as well as their capacity to process aberrant sensory input following injury and during chronic pain states.

Poster 247 – Tuesday 6th December

PROFILING SUBJECTIVE SYMPTOMS AND AUTONOMIC CHANGES ASSOCIATED WITH CYBERSICKNESS

Mr Alireza Mazloumi Gavgani, Dr Eugene Nalivaiko, Dr Karen Blackmore, Dr Keith Nesbitt

[1]*University of Newcastle*

Our aim was to expand knowledge of cybersickness – a subtype of motion sickness provoked by immersion into a moving computer-generated virtual reality. Fourteen healthy subjects experienced a 15-min rollercoaster ride presented via a head-mounted display (Oculus Rift), for 3 consecutive days. Heart rate, respiration, finger and forehead skin conductance were measured during the experiment; this was complemented by a subjective nausea rating during the ride and by Motion Sickness Assessment Questionnaire before, immediately after and then 1, 2 and 3h post-ride. Physiological measurements were analysed in three dimensions: ride time, association with subjective nausea rating and experimental day. Forehead, and to a lesser extent finger phasic skin conductance activity showed a correlation with the reported nausea ratings, while alteration in other measured parameters were mostly related to autonomic arousal during the virtual ride onset. A significant habituation was observed in subjective symptom scores and in the duration of tolerated provocation. The latter increased from 7.0±1.3 min on the first day to 12.0±2.5 min on the third day ($p<0.05$); this was associated with a reduced slope of nausea rise from 1.3±0.3 units/min on the first to 0.7±0.1 units/min on the third day ($p<0.01$). Furthermore, habituation with repetitive exposure was also determined in the total symptom score post-ride: it fell from 1.6±0.1 on the first day to 1.2±0.1 on the third ($p<0.001$). We conclude that phasic changes of skin conductance on the forehead could be used to objectively quantify nausea; and that repetitive exposure to provocative VR content results in habituation.

Poster 248 – Monday 5th December

VAGAL AFFERENT SIGNALING OF SMALL INTESTINE INFLAMMATION

Prof Robin Mcallen[1], Dr Bradford Bratton[1], Dr Anthony Shafton[1], Mr David Trevaks[1], Dr Tanya Kameneva[2], Dr Elma O'Sullivan-Greene[2], Prof David Grayden[2], Prof John Furness[1]

[1]*Florey Institute Of Neuroscience And Mental Health,* [2]*Dept of Electrical and Electronic Engineering*

It is generally believed that information concerning pain or injury to the intestine is signaled to the brain through spinal afferent neurons. However, it has been found that inflammatory cytokines can activate vagal afferents. Moreover, the vagus, perhaps reflexly via afferents, has anti-inflammatory effects on the gut. In order to resolve how intestinal inflammation is communicated to the brain, we recorded afferent fibre activity from vagal and mesenteric nerves in isoflurane-anaesthetized rats. A 6-10 cm segment of small intestine was inflamed by intraluminal application of trinitrobenzene sulphonate (TNBS, 1%). Direct afferent responses to this stimulus could be recorded from mesenteric nerves supplying that segment and from the celiac branch of the vagus. In both cases afferent unit activity increased rapidly, peaking after 1-2 minutes, followed by a raised level of discharge lasting at least 1 hour. Indirect afferent responses were recorded from the hepatic vagal branch: these built up slowly after a delay of 20-50 min. They occurred even when the inflamed gut segment had been denervated, so they must have been responding to a humoral signal, presumably carried to the liver by the portal circulation. Mathematical modelling of spike activity revealed two phases of vagal afferent signaling to the brain: an early phase signaled by vagal afferents that innervate the intestine, and a later phase signaled by both intestinal and hepatic vagal afferents. The latter phase is consistent with a response to inflammatory mediators. This composite neural signal may then drive anti-inflammatory reflex actions.

Poster 249 – Tuesday 6th December

SYSTEMATIC REVIEW AND META-ANALYSIS OF THE EFFICACY OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN ANIMAL MODELS OF STROKE: AN UPDATE

Dr Sarah K McCann[1], Fala Cramond[1], Prof Malcolm R Macleod[1], Dr Emily S Sena[1]

[1]*The University of Edinburgh*

Interleukin-1 receptor antagonist (IL-1RA) is an anti-inflammatory protein considered a promising candidate therapy for stroke. Here, we sought to update the existing systematic review and meta-analysis of IL-1RA in models of ischaemic stroke, published in 2009. Subsequent to this initial review, efforts have been made to improve the range and quality of preclinical evidence supporting IL-1RA to inform clinical application. We assessed efficacy, the range of circumstances under which efficacy has been tested, and whether the data appear to be confounded due to reported study quality and publication bias. We included 25 data sources, 11 additional to the original review. Overall, IL-1RA reduced our primary outcome measure, infarct volume, by 36.2% (95% confidence interval 31.6–40.7, $p<0.05$, n=76 comparisons, 1283 animals). Assessments for publication bias suggest 30 theoretically missing studies which reduce efficacy to 21.9% (17.3–26.4). Systematic reviews of preclinical studies are increasingly performed and can be used to identify gaps in a field and inform future experimental design, explain discrepancies between preclinical and clinical results, and inform clinical trial design. To our knowledge, this is the first update to a preclinical systematic review where the changes over time in a field can be charted and the possible impacts of systematic review on the directions taken by researchers investigated. We demonstrate that the preclinical data supporting IL-1RA has improved. Reporting of measures to reduce risks of bias has improved substantially and studies now include the use of animals with relevant comorbidities. The efficacy originally observed has been maintained.

Poster 250 – Monday 5th December

EXPLORING THE ROLE OF IRON IN DOPAMINERGIC CELL LOSS

Dr Gawain Mccoll[1], Ms Patricia Chege[1]

[1]The Florey Institute Of Neuroscience

Despite the prevalence of Parkinson's disease amongst our ageing population, an effective treatment beyond alleviating symptoms remains elusive. A limited understanding of the molecular basis of the disease has restricted the development of therapeutic strategies that directly target the major pathological feature of Parkinson's disease: the death of dopaminergic neurons.
Elevated brain iron has long been known to occur in Parkinson's disease. We propose that an iron–dopamine redox couple in the brain is an initiating chemical reaction preceding neuron death. Understanding the details of this damaging interaction may reveal how these two redox-active factors can be directly targeted through novel therapeutic intervention.
To further understand this interaction we are using the simplified dopaminergic network of the nematode, caenorhabditis elegans, to explore how and why dopaminergic neurons die. We have developed age-dependent models to explore the degenerative changes in dopaminergic cells following manipulation of iron homeostasis, endogenous dopamine synthesis and transport. Our data is consistent with a toxic interaction between dopamine and iron, and that this interaction can be targeted for intervention.
Reliance on toxin-based animal models for drug development may inadvertently be limiting identification of new therapeutics that will translate to effectively to the clinic. Our research suggests that rapid ageing animal models could identify therapeutic opportunities that target the toxic iron-dopamine couple.

Poster 251 – Tuesday 6th December

AN EXTENSIVE SOMATOSTATIN INTERNEURON NETWORK GATES VISCEROSENSORY SIGNALS IN THE SOLITARY TRACT NUCLEUS.

Dr Stuart McDougall[1], Prof Andrew Allen[2], Haoyao Guo[1], David Carter[2], Kimberly Thek[1]

[1]Florey Institute, Univeristy of Melbourne, [2]Dept. Physiology, Univeristy of Melbourne

The solitary tract nucleus (NTS) is the termination site of vagal afferent sensory neurons. Inhibitory input at the NTS is thought to alter autonomic reflex performance. Here we quantified the impact somatastatin (SOM) interneurons have on vicerosensory signal throughput within the NTS. Using a SOM-Channel rhodopsin 2-YFP mouse model, we took whole cell recordings from NTS neurons in horizontal brain stem slices that contained both the NTS and solitary tract (ST, the sensory afferent bundle). We recorded neurons randomly to determine if they received ST input and/or SOM input. LED pulses evoked consistent action potential dependent GABAergic and glycinergic IPSCs in recorded neurons (n=42). LED-evoked IPSC amplitude increased with LED duration indicating converging inhibitory input at these NTS neurons. Combined, these data indicate SOM input to NTS neurons is exclusively inhibitory and that though SOM neurons are relatively sparse, SOM efferents synapse extensively throughout the NTS. Shocks to the solitary tract evoked low jitter EPSCs that identified second order NTS neurons. We demonstrated that almost all randomly sampled neurons were second order. In current clamp studies, we determined the impact of SOM input on action potential (AP) generation and throughput. LED-IPSPs prevented spontaneous AP firing when neurons were depolarized and delayed AP onsets in response to current injections. LED-IPSPs also prevented ST-evoked AP firing, effectively dropping throughput from 86.7 to 6.7%. Combined, these data indicate that an extensive SOM interneuron network exists within the NTS that likely operates to coordinate or alter autonomic reflex function.

Poster 252 – Monday 5th December

A TACTILE DIRECTION TEST TARGETING SLOWED NERVE CONDUCTION

Dr Sarah McIntyre[1,2], Mr Gustav Bergström[2,3], Dr Richard M Vickery[2,4], Dr Ingvars Birznieks[1,2,4], Dr Paul P Breen[1]

[1]MARCS Institute for Brain, Behaviour and Development, Western Sydney University, [2]Neuroscience Research Australia, [3]Faculty of Medicine and Health Sciences, Linköping University, [4]School of Medical Sciences, UNSW Australia

Peripheral neuropathy is associated with a decline in tactile sensation; typically measured as detection of a brief touch or vibration applied to the skin. Evoked peripheral afferent activity, if relayed to the central nervous system, is sufficient to perform these simple tasks. Therefore task performance reflects mainly axonal loss caused by neuropathy. A more complex task affected by axonal conduction velocities may enable early detection of neuropathy and distinguishing different underlying causes. Judging the direction of apparent motion, where neighbouring skin locations are successively tapped, requires the observer to correctly order the sequential touch events in space and time. Performance on this task is likely affected by the increased dispersion in conduction velocities associated with peripheral neuropathy. We developed an apparent motion device for use in clinical settings. Baseline data were collected from healthy participants (12 female, 9 male, aged 21 - 76). Four tactors 20 mm apart tapped the skin. An adaptive staircase method was used to find the direction discrimination threshold, defined as the time between taps at which direction discrimination was 80% correct. The median threshold on the foot was 15 ms (inter-quartile range: 10 – 26 ms); on the lower leg it was 61 ms (IQR: 23 – 97 ms). On the foot, performance was better for motion in the proximal direction (median 88% correct) than in the distal direction (median 78% correct; $N=21$, $W^+ = 194.5$, $p < 0.001$). This directional difference may be sensitive to conduction velocity, and should be accounted for when testing clinical populations.

Poster 253 – Tuesday 6th December

THE RELATIONSHIP BETWEEN GAMMA AMINOBUTYRIC (GABA) CONCENTRATION IN VISUAL CORTEX, PERCEPTUAL RIVALRY, AND DURATION POST MIGRAINE EVENT.

Allison McKendrick[1], Yu Man Chan[1], Kabilan Pitchaimuthu[1], Qhi-Zu Wu[4], Olivia Carter[2], David Badcock[3], Gary Egan[4]

[1]Department of Optometry & Vision Sciences, The University of Melbourne, [2]Melbourne School of Psychological Sciences, The University of Melbourne, [3]School of Psychology, University of Western Australia, [4]Monash Biomedical Imaging, Monash University

Migraine is a very common neurological disorder that involves vision. It is hypothesised that an imbalance between cortical inhibition and excitation may explain a range of the visual anomalies present in migraine, even at times in between migraine events. Our aim was to determine whether perceptual performance in migraine is related to the concentration of the inhibitory neurotransmitter Gamma Aminobutyric Acid (GABA) concentration in visual cortex, and additionally whether an important covariate is duration post-migraine. Seventeen people with migraine participated (aged 21-49: 9 with aura, 8 without aura). GABA concentration was estimated using MEGA PRESS in a Siemens Skyra 3T scanner with 32-channel head coil. GABA/tCr was adjusted for grey matter volume. Binocular rivalry was measured for red/green gratings of 1.5 c/deg oriented at 45/135 deg in 5 runs of 90 sec each on the same day. Duration post-migraine at the time of testing was between 1 and 60 days. Both GABA concentration in visual cortex and median percept duration for the rivalry task were positively correlated with duration post-migraine (GABA: r=0.610, p=0.009; Rivalry: r=0.547, p=0.023). There was a trend for GABA concentration to be correlated with slower rivalry switch rate (longer median percept duration) consistent with previous reports in non-migrainous groups (r=0.456,p=0.066: van Loon et al., 2013). Our data suggests that duration post-migraine influences the level of GABA-ergic inhibition in visual cortex.

Poster 254 – Monday 5th December

THE MESOSCALE CONNECTOME OF SYMPATHETIC PREMOTOR NEURONS OF THE RAT ROSTRAL VENTROLATERAL MEDULLA

Dr Bowen Dempsey[1], Dr Sheng Le[1], Dr Anita Turner[1], Dr Radhika Ramadas[1], Prof Jan Bjaalie[3], Dr Clement Menuet[2], Dr Rachael Neve[4], Prof Andrew Allen[2], A/Prof Ann Goodchild[1], **Dr Simon McMullan[1]**

[1]Macquarie University, [2]University of Melbourne, [3]University of Oslo, [4]McGovern Institute for Brain Research

Spinally projecting neurons in the rostral ventrolateral medulla (RVLM) play a critical role in the generation of vasomotor sympathetic tone; the factors that determine their ongoing activity have for several decades remained an unresolved core issue in the field of autonomic neuroscience. In this study we use a genetically restricted viral tracing strategy to definitively map their spatially diffuse connectome.
Our strategy was first to use a recombinant herpes vector with a retrograde transduction profile to drive the expression of TVA and rabies glycoprotein in neurons that project to the interomediolateral cell column of the spinal cord, a major site of termination of sympathetic premotor neurons. We then infected TVA- and rabies glycoprotein-expressing RVLM neurons with glycoprotein-deleted rabies. This enabled us to map brain-wide sources of monosynaptic input to bulbospinal RVLM neurons using MRI-based volumetric atlas of the Sprague Dawley rat brain and a novel image alignment and visualization tool.
We identified prominent inputs from well-established neurohumoral and viscero-sympathetic actuators and inputs that spanned medullary autonomic and respiratory nuclei, as well as supramedullary autonomic nuclei. The majority of inputs lay within the brainstem (92% (88 – 94)), with 50% residing within 2.5 mm (2.3 – 2.7) of the RVLM epicenter, with a pronounced (74% (75 – 80)) ipsilateral bias. Our data support a hitherto under-appreciated input from local medullary neurons and a relatively minor input from supra-medullary structures such as the hypothalamus, thalamus and midbrain. This organizational scheme aligns with recently proposed hypothetical connectivity models from other systems.

Poster 255 – Tuesday 6th December

LASER CAPTURE MICRODISSECTION (LCM) TO ISOLATE HIGH QUALITY BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND TROPOMYOSIN RECEPTOR KINASE B (TRKB) RNA FROM PURE POPULATIONS OF RAT SPINAL CORD MOTOR NEURONS

Dr Prachi Mehta[1], Mr Brian Premkumar[1], Dr Renee Morris[1]

[1]UNSW Australia

Objective:
The mammalian central nervous system (CNS) is composed of multiple cellular elements, making it challenging to segregate one particular cell type to study their gene expression profile. As motor neurons represent only 5–10% of the total cell population of the spinal cord, a meaningful transcriptional analysis of these neurons is impossible to achieve from homogenized spinal cord tissue. We used LCM technique to identify and isolate RNA from pure populations of spinal cord motor neurons in naive and rubrospinal tract- (RST) transected rats.

Method:
Spinal cord segments C2-C3 and C4-C5 were dissected out from naive and RST-transected rats. These segments were fast freeze with liquid nitrogen and the tissue blocks were sectioned to produce 50 µm sections mounted on RNase-free slides. The tissues were then stained

with Azure B to identify the motor neurons and to capture them using a LCM system. RNA was isolated and RNA integrity (RIN) was determined. RT-PCR analysis was performed to confirm the presence of microdissected transcripts. Spinal cord homogenate was used as a control.

Key Findings:
RNA Integrity Numbers (RINs) for all tissue samples were above 8, which indicates intact, high quality RNA. RT-PCR analysis revealed that BDNF and TrkB transcripts were present in all motor neuron samples, indicating that the levels of these gene transcripts are not affected by the loss of RST supraspinal inputs.

Conclusion:
The isolation of pure populations of neurons with LCM techniques allows for robust transcriptional characterization that cannot be achieved with spinal cord homogenates

Poster 256 – Monday 5th December

IN VIVO TOLERABILITY AND EFFECTS OF AUTOPHAGY ENHANCERS AS POTENTIAL THERAPEUTIC INTERVENTIONS FOR ALZHEIMER'S DISEASE

Stephanie Mercer[1], Ming Yang Isaiah Cheong[1], Professor Wickliffe Abraham[1], Dr Stephanie Hughes[1]

[1]University Of Otago

Dysfunction in the autophagy-lysosomal pathway is implicated in Alzheimer's disease (AD) pathophysiology. In mouse models, enhancement of autophagy through transcription factor EB (TFEB), a "master regulator" of genes for lysosomal biogenesis and autophagy, reduces plaque pathology and partially reverses behavioural deficits. Pharmaceutical agents such as β-cyclodextrin and fibrates (Gemfibrozil and Fenofibrate), approved for clinical use in other indications, were found to increase brain levels of TFEB and downstream markers of autophagic function, although the doses required to achieve this effect chronically *in vivo* are unknown. Hence, the present study aimed to first examine the tolerability of these three drugs in wild-type mice and examine whether these doses were sufficient to elevate autophagy. Mice were administered orally a low or medium dose of one drug twice daily, three times per week for four weeks. There were no qualitative pathological changes to the kidneys or liver across the treatment groups, or any other evidence of drug intolerability. In the brain, grouped *t*-tests for each drug dose showed increased levels of SQSTM1 and LAMP-2 in dorsal cortical, hippocampal and thalamic regions for medium doses of β-cyclodextrin and low doses of Fenofibrate relative to vehicle controls, (p all < 0.05) suggesting increased targeting of cellular waste and increased autophagosome-lysosome fusion. TFEB protein levels and localisation, and substrate degradation (Cathepsin D) will now be examined to determine effects across the autophagic process. The drug and dose that best enhances autophagy will be tested for therapeutic efficacy in a mouse model of AD.

Poster 257 – Tuesday 6th December

PHARMACOGENETIC STIMULATION OF NEURONAL ACTIVITY INCREASES MYELINATION IN AN AXON-SPECIFIC MANNER

Dr Stanislaw Mitew[1], Dr Ilan Gobius[2], Ms Laura Fenlon[2], Dr Stuart McDougall[3], Dr David Hawkes[4], Dr Yao Lulu Xing[1], Ms Helena Bujalka[5,6], Prof. Andrew Gundlach[3], Prof. Linda Richards[2], Prof. Trevor Kilpatrick[3,5], Dr Ben Emery[3,5,6], **Dr Tobias Merson[1]**

[1]Australian Regenerative Medicine Instituite, [2]Queensland Brain Institute, [3]The Florey Institute of Neuroscience and Mental Health, [4]Department of Pharmacology and Therapeutics, [5]Department of Anatomy & Neuroscience, [6]Jungers Center for Neurosciences Research

Increasing evidence suggests that neuronal activity influences myelination in an adaptive manner, allowing for strengthening or synchronisation of specific connections and circuits. Nevertheless, the predominant cellular components of the adaptive myelination response in the intact brain remain to be fully elucidated. We investigated this by manipulating neuronal activity in the postnatal mouse brain using a pharmacogenetic approach (the DREADDs). Enhancement of neuronal activity increased the proliferation and subsequent differentiation of OPCs within the white matter of both the developing and adult CNS, albeit with slower kinetics in the adult. In addition to these relatively broad lineage changes, neuronal activity resulted in selective changes to the myelination of activated axons, within increased thickness of the myelin surrounding DREADD expressing axons and preferential myelination of these axons by newly formed oligodendrocytes. Conversely, attenuating neuronal activity via overexpression of Kir2.1, an inward rectifying potassium channel, resulted in reduced selection of axons for myelination during early postnatal development. Our data demonstrate that the level of electrical activity within axons serves a critical role in defining their propensity to be selected for myelination. Further, the study reveals that increasing neuronal activity exerts diffuse effects that increase the pool of myelinating oligodendrocytes and specific effects acting at the level of the axon which increase both the likelihood that active axons are selected for myelination and the thickness of myelin that ensheathes them. Collectively, these data provide a deeper understanding of the cellular mechanisms underlying adaptive myelination within the mammalian central nervous system.

Poster 258 – Monday 5th December

MORPHOLOGY OF DONOR & RECIPIENT NERVES UTILISED IN NERVE TRANSFERS TO RESTORE UPPER LIMB FUNCTION IN CERVICAL SPINAL CORD INJURY.

Mary P Galea[12,] **Aurora Messina[1],** Natasha van Zyl[3], Andrew Nunn[2], Catherine Cooper[4], Jodie Hahn[4]

1: Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Parkville VIC 3010, Australia
2: Victorian Spinal Cord Service, Austin Health, Studley Road, Heidelberg VIC 3084, Australia
3: Department of Surgery, Austin Health, Studley Road, Heidelberg VIC 3084, Australia
4: Department of Occupational Therapy, Austin Health, Studley Road, Heidelberg VIC 3084, Australia

Loss of hand function after cervical spinal cord injury (SCI) has an enormous impact on independence. Multiple nerve transfer surgery has recently been applied successfully after cervical SCI to restore some critical arm and hand functions. Although the integrity of nerves is assessed using muscle strength testing and intramuscular EMG, these cannot show the extent and the manner in which donor and recipient nerves/muscles respond to injury.
Objective: In this study we directly assess the morphology of nerves that available for biopsy at the time of surgery. Our objective was to document morphologic features observed from specimens above and below the spinal injury level.
Methods: Twenty nerve samples were collected from 3 patients at the time of surgery, fixed in 2.5% Glutaraldehyde, processed and embedded in Araldite Epon. Semi-thin sections were cut, mounted onto slides, and stained with methylene or toluidine blue for light microscopy.
Results. Approximately, 80% of nerves showed abnormalities. Most common were myelin thickening and folding, demyelination, inflammation and a reduction of large myelinated axon density. Less commonly observed included a thickened perineurium, oedematous endoneurium and Renaut bodies. Significantly, very thinly myelinated axons and groups of unmyelinated axons were observed in all nerves.
Conclusions. Abnormalities exist in most donor and recipient nerves although some differ in appearance and possible aetiologies. Regenerative efforts were noted in many nerve specimens. Abnormalities observed may be preventable or reversible.

Poster 259 – Tuesday 6th December

METABOLIC STATUS-DEPENDANT PLASTICITY OF POMC NEURONS IN THE ARCUATE NUCLEUS

Dr Natalie Michael[1], Dr Marco van den Top[3], Dr Stephanie Simonds[1], Professor Michael Cowley[1], Professor David Spanswick[3]

[1]Metabolic Disease and Obesity Program, Biomedicine Discovery Institute, Department of Physiology, Monash University, [2]Neuroscience Program, Biomedicine Discovery Institute, Department of Physiology, Monash University, [3]Neurosolutions Ltd.

Proopiomelanocortin (POMC) neurons of the hypothalamic arcuate nucleus (ARC) are critical regulators of energy homeostasis. Whilst their role in regulating food intake and energy metabolism is extensively recognised, little is known of the fundamental electrophysiological properties of these neurons, how they function to integrate and encode information regarding energy status, and how they formulate output to drive appropriate changes in behaviour and the internal environment. Here we used whole-cell patch clamp electrophysiology to record eGFP-expressing POMC ARC neurons in adult mouse hypothalamic brain slices prepared from animals fed *ad libitum*, fasted, or fed high-fat diet (HFD). Passive membrane properties and active threshold and sub-threshold conductances were characterised. The afterhyperpolarisation (AHP) following action potential discharge varied in POMC neurons and subthreshold active conductances were differentially expressed, including: low-threshold T-type calcium conductance (I_T); hyperpolarisation-activated time-dependent inward rectification (I_h); instantaneous inward rectification (I_{IR}); and transient outward rectification (I_{TR}). Expression of I_T increased in POMC cells prepared from fasted animals, with increased incidence of cells displaying this conductance compared to control fed and HFD groups. Resting input resistance was significantly increased ($p < 0.01$, compared to fed and fasted) and expression of I_{IR} decreased in HFD-POMC neurons. The proportion of POMC neurons that were active increased with HFD and was accompanied by a significant increase in firing rate ($p < 0.05$, compared to fed and fasted conditions). These results suggest POMC neurons are heterogeneous and subject to metabolic-status-dependent functional plasticity: adapting intrinsic electrical excitability with short- and long-term changes in energy status.

Poster 260 – Monday 5th December

MMN AND GAMMA OSCILLATION CHANGES WITH ACUTE DOSES OF AN NMDAR ANTAGONIST IN AN ANIMAL MODEL

Emeritus Professor Patricia Michie[1,2,3], Dr Lauren Harms[1,2,3], Dr. W Ross Fulham[1], Dr Aaron Wong[1], Associate Professor Juanita Todd[1,2,3], Professor Ulrich Schall[1,2,3], Professor Deborah Hodgson[1,2]

[1]University of Newcastle, [2]Centre for Brain and Mental Health Research, [3]Schizophrenia Research Institute

1. Two electrophysiological indices that are changed in schizophrenia are (i) mismatch negativity (MMN) to rare deviant auditory stimuli, the amplitude of which is reduced and (ii) oscillatory activity in the gamma range that is reduced when driven by auditory steady state stimuli but increased in the spontaneous state in patients. Both indices are thought to be dependent on glutamate n-methyl-d-aspartate receptor (NMDAR) function. Dysfunction of the glutamatergic system has been most strongly implicated in the cognitive deficits that are core to schizophrenia. We examined whether MMN-like responses and oscillatory activity following administration of an NMDAR antagonist, MK-801, in an animal model showed schizophrenia-like changes consistent with glutamatergic involvement.
2. Screw electrodes were implanted in Wistar rats and EEG recorded using a wireless system. Auditory event-related potentials to frequency deviants were measured in two flip-flop oddball paradigms that allowed matched stimulus characteristics and in a many-standards paradigm that controlled for adaptation effects. MMN-like responses reflecting deviance detection were extracted by comparing ERPs to oddball with control deviants. Auditory steady state responses (ASSRs) to auditory click trains of 10 - 50Hz and spontaneous (unstimulated) activity in the gamma range were extracted.

3. Both early and late components of ERPs exhibited deviance detection but only late components were reduced following high doses of MK-801. ASSR power and phase-locking to 30-50Hz trains were reduced but spontaneous gamma activity was increased by MK-801.

Alterations to MMN-like potentials and oscillatory activity in this animal model are consistent with proposed role of the glutamatergic system in schizophrenia

Poster 261 – Tuesday 6th December

DIFFERENTIAL EXPRESSION OF GABA-A RECEPTOR ALPHA1, ALPHA2 AND ALPHA3 SUBUNIT IN SUBCELLULAR FRACTIONS.

Ms Stephanie Miller[1], Professor Paul Colditz[1], Dr Tracey Bjorkman[1]

[1]Perinatal Research Centre, UQ Centre for Clinical Research, The University Of Queensland

OBJECTIVE: The $GABA_A$ receptor ($GABA_AR$) is pentameric ligand-gated ion channel, comprised of various subunit isoforms, with the majority of functional receptors containing 2□□2β-1□ subunit. The predominant □-isoforms are $□_1$, $□_2$ and $□_3$; of which $□_3$ is most prevalent in the developing brain. With age $□_1$ expression increases, such that $□_1$ is the predominant □-subunit expressed in adult synaptic $GABA_AR$ in various brain regions. We have previously observed changes in total protein expression of the $□_1$ and $□_3$ subunits after hypoxia-ischaemia and seizures in the neonatal pig brain. We aimed to further explore the subcellular distribution of these □-subunits, in our model of perinatal asphyxia. METHODS: Using our established model of perinatal asphyxia in the neonatal pig (n=13), brain tissue was collected post-mortem from control and hypoxia-ischaemic animals at 72h-post asphyxia. Tissue was homogenised and fractionated into nuclear, cytosolic and membrane fractions, Western blot analysis was performed to investigate protein expression. RESULTS: Highest expression was observed in the nuclear and membrane fractions compared with cytosol for all □-subunits investigated. There was no expression of $□_3$ in the cytosolic fraction however in a subset of animals, a smaller band was identified in the cytosolic fraction. CONCLUSION: Distribution of the □-subunits predominantly to the membrane and nuclear fractions suggest involvement in receptor function and trafficking.

Poster 262 – Monday 5th December

DEFINING THE BOUNDARIES OF HIPPOCAMPAL AREA CA2 IN ADULT AND DEVELOPING MICE

Mr Amer Mitchelle[1], Ms Jade Pham[2], Professor Charles Watson[3]

[1]Faculty of Medicine, Notre Dame University, [2]Neuroscience Research Australia, [3]Faculty of Health Sciences, Curtin University

The CA2 area of the hippocampus can be defined by a small region of gene expression not shared with the rest of the hippocampus. Using a CA2 specific mouse Cre lineage, Hitti and Siegelbaum (Nature 508:88, 2014) showed that deletion of the CA2 area of the hippocampus resulted in loss of social recognition, but did not damage spatial and contextual memory.
While the CA1-CA2 boundary is relatively easy to identify, some stains and gene expression patterns suggest a range of possible locations for the boundary separating CA2 from CA3. We have examined the boundaries revealed by a range of histological and immunohistochemical stains, gene expression, and high resolution MRI in mice and rats in order to define a standardised CA2-CA3 boundary that could be used in future experimental studies. We found that acetylcholinesterase staining is the most satisfactory CA2-CA3 boundary marker, and that this boundary could be recognised in MR images.
Social recognition memory in mice is not fully developed until about 3-4 weeks of age. We searched gene expression atlases to determine whether there is a correlation between the onset of this behaviour and the maturation of gene expression in CA2 in mice. We found that there is a good correlation between the postnatal maturation of expression of Pcp4, AChE, and Avpr1B (7-14 days) genes in CA2 and the onset of species recognition memory in mice (14-28 days).

Poster 263 – Tuesday 6th December

UNDERSTANDING THE DYNAMICS OF OLIGODENDROCYTE TURNOVER IN HEALTH AND DISEASE

Dr Stan Mitew[1,2], Dr Kaylene Young[3], Dr Ben Emery[4], Dr Tobias Merson[1]

[1]The Florey Institute Of Neuroscience And Mental Health, [2]Department of Anatomy and Neuroscience, University of Melbourne, [3]Menzies Institute for Medical Research, [4]Jungers Center for Neuroscience Research, Oregon Health and Science University

Although the majority of CNS oligodendrocytes (OLs) are generated during early development, significant levels of oligodendrogliogenesis persist into adulthood. It is not currently known if adult-born OLs are functionally similar to developmentally generated OLs.

Using healthy adult *PLP-CreER^{T2}: Rosa26-EYFP* mice, we sought to determine how many OLs are lost and replaced by new OL progenitor cells over the course of a year. We found close to half of the mature OLs that were genetically labelled at postnatal day 60 (P60) in the corpus callosum had died and were replaced by P360. Similarly, in the optic nerve, some 29% of all OLs had been turned over. Importantly, we saw no changes in the total OL density in either region for the duration of the study. We also assessed the morphological characteristics of myelin internodes produced by newly-differentiated OLs that were added to the CNS during development, healthy adulthood and in response to demyelination. In *PDGFRa-CreER^{T2}: Tau-mGFP* mice receiving a 0.2% cuprizone diet for 6 weeks to induce demyelination, there was a robust proliferative response leading to an increase in newly-generated oligodendrocytes. Interestingly, we found that while remyelinating oligodendrocytes had fewer internodes than similarly aged oligodendrocytes in healthy adult controls, their internodes were

significantly longer, producing a similar total myelin output. Collectively, adult-born oligodendrocytes had much shorter internode lengths compared to developmentally-born oligodendrocytes.

In conclusion, our results emphasise the high degree of plasticity within white matter tracts in the adult CNS and highlight the difference between myelin produced in development and adulthood.

Poster 264 – Monday 5th December

α9-NICOTINIC ACETYLCHOLINE RECEPTORS PLAY A ROLE IN AFFECTIVE BEHAVIOUR AND STRESS PHYSIOLOGY

Dr Sarasa Mohammadi[1], Dr Thomas Burton[1], Professor MacDonald Christie[1]
[1]The University Of Sydney

Objectives: The α9 subunit of nicotinic acetylcholine receptors (α9-nAChRs) has been implicated in chronic pain, and is gaining interest as a target for novel analgesics. However, the potential for adverse effects due to blocking α9-nAChRs for pain relief are not yet known. Expression of α9-nAChRs in the HPA-stress axis suggests they play a role in stress responses, but this has not been widely explored. Here we investigate the role of α9-nAChRs in affective behavioural and stress physiology in mice
Methods: We compared wildtype with α9-nAChR-knockout genotypes in tests of affective behaviours (elevated plus maze, forced swim test, sucrose preference) and measured corticosterone responses to stress. Non-stressed and sub-chronically restraint-stressed conditions were compared. Additionally, circadian patterns, and responses to sucrose-reward and non-reward were observed in the homecage using the IntelliCage system.
Key findings: Deletion of α9-nAChRs caused no observable changes in affective behaviours in non-stressed mice, compared to wildtype mice. However, sub-chronic stress caused significantly decreased stress-induced arousal ($P < 0.05$) and increased anxiety-like behaviour ($P < 0.05$) in the absence of α9-nAChRs. Physiological responses to acute stress were muted ($P < 0.05$) in non-stressed knockout mice, but exaggerated ($P < 0.01$) after sub-chronic stress. In the homecage, knockout mice exhibited altered circadian patterns of activity ($P < 0.0001$) and anhedonia was observed in knockout mice after non-reward, indicative of extinction-induced depression ($P < 0.0001$).
Conclusion: We have uncovered a novel role of α9-nAChRs in stress responses wherein α9-nAChRs contribute to the regulation of affective state in situations of sub-chronic stress and loss of reward. Consequently, disruptions to such regulation must be considered prior to pharmacological inhibition of α9-nAChRs for clinical treatments of pain.

Poster 265 – Tuesday 6th December

STEREOLOGICAL ESTIMATES OF THE MOTOR NEURON POPULATIONS CAUDAL TO A RUBROSPINAL TRACT (RST) TRANSECTION IN THE RAT

Mr Rahul Mohan[1], Mr Brandon Mark Wild[1], Dr Renee Morris[1]

[1]Translational Neuroscience Facility, Unsw Medicine, Unsw Australia

Background: One significant outcome of a spinal cord injury is the abolishment of the supraspinal input to the motor neurons below the lesion, therefore resulting in paralysis. The majority of research has focused on understanding the pathophysiological events surrounding a lesion, however, there is little knowledge regarding what changes occur to motor neurons below a lesion. Therefore, the aim of this project is to determine the morphological changes to motor neurons below a spinal cord injury.
Method: Rats were subjected to a unilateral partial transection at C3-4 spinal cord segments. Animals were sacrificed one, three, seven and fourteen days post-lesion and the C5-6 and L2-3 spinal cords segments were dissected and histologically processed. Using the Optical Fractionator workflow component of the Stereo Investigator software, the morphology and number of motor neurons were quantified.
Results: Analysis revealed that following injury, the motor neurons at both C5-6 and L2-3 did not undergo cell death or atrophy. No significant changes were detected in the motor neuron populations both ipsilateral and contralateral to the injury at both sites across all time points of investigation.

Conclusion: A unilateral partial transection of the spinal cord results in no detectable changes to the motor neuron populations below a lesion. The absence of any quantifiable changes carries the implications that these cells are viable and available for the application of potential therapeutic interventions. This knowledge is critical for future therapeutic scenarios that aim to restore the function of motor neurons below an injury.

Poster 266 – Monday 5th December

THE ROLE OF THE RUBROSPINAL TRACT (RST) IN OVER GROUND LOCOMOTION IN THE RAT.

Morris R[1], Brown JS[1], Robinson JH[1]

1. Translational Neuroscience Facility, School of Medical Sciences, UNSW Medicine, UNSW, Sydney, Australia.

We have shown that unilateral lesions that exclusively disrupt the rubrospinal tract (RST) at cervical levels abolish the ability to pronate the wrist in a lateral to medial motion, a motion that allow the sequential placement of the digits over a food object during skilled reaching (Morris et al., 2011). This movement is termed *arpeggio* because it is similar to the movement of the fingers of a piano player performing

an arpeggio scale. Interestingly, naïve rats also perform the *arpeggio* while walking. The aim of the current study was to determine whether the RST also controls this *locomotive arpeggio*. Female Long Evans rats were trained to walk in a straight alley and their performance was video recorded (baseline), after which they were subjected to unilateral cervical RST transection. Seven days later, the rats were placed in the alley again and their post-surgical performance was also video recorded. The locomotive sequence during alley walking was found to be comprised of four distinct movement elements: *limb lift and release, carry, advance* and *arpeggio*. Frame-by-frame analysis of the video recordings revealed that RST transection not only impairs skilled reaching *arpeggio* but also disrupts the *locomotive arpeggio*. Locomotive abilities can be evaluated with the same movement scale in both spinal cord-injured rats and patients, therefore allowing clinicians to draw invaluable information from pre-clinical investigations. It is our opinion that an effective treatment that involves the functional restoration of the RST would result in the return of the *arpeggio* movement in both reaching and walking.

Poster 267 – Tuesday 6th December

MODELLING DRAVET SYNDROME USING MICRO-ELECTRODE ARRAYS

Ms Emma Morrisroe[1], Ms Dulini Mendis[2], Dr Snezana Maljevic[1], Dr Elena Gazina[1], Professor Saman Halgamuge[2], A/Prof Christopher A Reid[1], Professor Steven Petrou[1]

[1]Florey Institute Of Neuroscience & Mental Health, [2]University of Melbourne

Dravet Syndrome (DS) is a severe form of childhood epilepsy with limited treatment options. Although single-cell and whole animal models have revealed many aspects of the underlying pathology they do not lend themselves to scalable drug discovery. Micro-electrode arrays (MEAs) can be used to characterise network scale pathology useful for diagnosis and drug discovery. Here, we first aimed to use MEA analysis of cultured DS neurons to identify network scale signatures as a prelude to future drug discovery studies.

Methods: Cortical neurons were dissected from both heterozygous Scn1a(R1407X) mice and their WT littermates and plated on MEAs. After four weeks in culture, network activity was recorded and 37 different activity parameters were extracted.

Results: At day 28, 12 of 37 activity parameters differed between HET and WT. It was seen that the heterozygous cultures had, amongst other parameters, a lower coefficient of variation for the parameters single channel burst durations ($p<.001$), single channel burst sizes ($p<.01$), and network burst durations ($p<.001$).

Conclusions: We discovered a network scale signature that differentiated DS cultures from controls. Moreover, changes in activity parameters were consistent with increased synchronicity a characteristic expected of epileptic networks. Further studies will involve screening a range of anti-epileptic drugs against this pathological signature. These studies provide a framework for analysis and drug discovery in other epileptic and neurogenetic disorders.

Poster 268 – Monday 5th December

METHYLATION STATUS AND LINE-1 EXPRESSION IN AGING RAT BRAIN

Dr Somnath Mukherjee[1], Prof. KC. Upadhyaya[1], Prof. Deepak Sharma[1]

[1]Jnu

Objectives: LINE 1 is an autonomous, non-LTR retrotransposon and the L1 retrotransposons constitute around 17%, of the human, mouse and rat genomes respectively. Under normal physiological conditions, the retroelements remain by and large transcriptionally silent but are activated in response to biotic and abiotic stress conditions. Our objectives were to study the transcriptional expression of L1Rn elements in different age group brain regions and correlate with corresponding DNA methylation levels.

Methods: Real time PCR analysis using RNA isolated from various brain regions and various tissues from old and young wistar rats was carried out to determine the change in L1 transcripts. DNA methylation assay was performed using COBRA method.

Results: There was no significant change in the expression of L1Rn in various brain regions of 2 month old and 18 month old rats except cerebral cortex.

Conclusion: In conclusion, the degree of hypomethylation in promoter CpG islands in LINE-1 repetitive sequences do play essential role in LINE-1 element expression. Besides tissue specific factors do play pivotal role in LINE-1 expression.

Poster 269 – Tuesday 6th December

DYNAMIC SPIKING PROPERTIES OF NEURONS IN THE LATERAL GENICULATE NUCLEUS OF MARMOSETS

Mr Brandon Munn[1,2], Mrs Natalie Zeater[2,3,4], Dr Sam Solomon[4,5], Dr Soon-Keen Cheong[3,4], Dr Pulin Gong[1,2], Professor Paul Martin[1,2,3]

[1]School of Physics, The University Of Sydney, [2]ARC Centre of Excellence for Integrative Brain Function, [3]Save Sight Institute, [4]School of Medical Sciences, The University of Sydney, [5]Department of Experimental Psychology, University College

Objective: In absence of patterned visual stimuli, LGN neurons fire quite irregularly. We here analyse the dynamics of spiking activity in LGN neurons and compare these dynamics with those described in cortical neurons.
Method: Extracellular action potentials of visually-responsive cells were recorded in sufentanil-anaesthetised marmosets using a Neuronexus (16x2) silicon array probe. The visual stimulus consisted a uniform grey 20 deg. field, intensity ~50 cd/m2. The instantaneous firing rate and variability relative to Poisson process (Fano factor) over time windows between 0.1 s and 100 s were calculated.
Results: The Fano factor of parvocellular (P, n =10/12), magnocellular (M, n=5) and koniocellular (K, n=26/29) neurons is close to unity for time windows less than 1 s (i.e. Poisson-like property) but rises monotonically with window width for time windows > 10 s (Scale-free/Fractal property). Transition from Poisson-like to Fractal behaviour occurs at shorter time windows for K cells than for P and M cells ($p < 0.01$, Kruskal-Wallis test). Further, the distribution of firing rates of all LGN neurons follows a log-normal distribution, instead of the Gaussian distribution that is commonly assumed.
Conclusion: Maintained firing activities of LGN neurons exhibit non-Gaussian dynamics, which are comparable to those described for cortical neurons (Churchland et al., Nat. Nsci, 2010). This similarity suggests that cortical dynamics may have thalamic origins, and that to get a fundamental understanding of brain dynamics, an integrative investigation of thalamocortical interaction is required.

Poster 270 – Monday 5th December

ASSESSMENT OF TWO NUTRITIONAL SUPPLEMENTS FOR THE PREVENTION OF POST-CONCUSSIVE SYNDROME

Sabrina Salberg[1], Sydney Candy[1], Katrina Yu[1], **Richelle Mychasiuk[1]**

[1]University Of Calgary

Traumatic brain injury (TBI) is a leading cause of death and disability, with mild TBI comprising the greatest proportion of this endemic. While many recover quickly, a significant proportion suffer from post-concussive syndrome (PCS). The ambiguity in prognosis is likely due to pre-existing differences that render some individuals susceptible to PCS and others resilient; understanding this heterogeneity is an important question that may permit the generation of preventative strategies to eliminate PCS. Research has demonstrated that dietary manipulations influence neurological health and psychological wellbeing. This research project aimed to utilize two dietary supplements to decrease susceptibility to concussion and prevent PCS. One supplement combined resveratrol, prebiotic fiber, and omega-3 fatty acids (3S) while the other added only prebiotic fiber to the diet (1S). Male and female rats were randomly assigned to one of 6 experimental conditions: 3S-TBI; 3S-Sham; 1S-TBI; 1S-Sham; Placebo-TBI; and Placebo-Sham. A behavioral test battery designed to examine PCS was administered beginning 24hrs post-TBI and continued for 17 days. Animals were sacrificed and tissue from the prefrontal cortex and hippocampus was harvested to examine changes in expression of 6 genes (*Aqp4, Bdnf, GFAP*, *Maoa*, *Sirt1, Tau*). While all animals that received an injury exhibited acute symptoms validating the presence of a concussion, animals in the 3S group, unlike animals in the 1S and Placebo group, failed to demonstrate behavioural impairments consistent with PCS. Gene expression changes corroborate these findings. Treatment with the 3S supplement appears to have prevented the onset of PCS in animals that received a TBI.

Poster 271 – Tuesday 6th December

EFFECTS OF PRENATAL HYPOXIA ON AMYLOID B-CLEARING PROTEINS AND SYNAPTIC PLASTICITY IN RAT BRAIN

Dr Natalia N Nalivaeva[1,2], Dr Nadezhda M Dubrovskaya[2,3], Dr Olga S Alekseeva[2], Dr Dmitrii S Vasilev[2,3], Dr Igor A Zhuravin[2,3], Prof Anthony J Turner[1]
[1]School of Biomedical Sciences, University of Leeds, [2]I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of RAS, [3]Saint-Petersburg State Pediatric Medical University

Pathogenesis of the sporadic form of Alzheimer's disease is connected with impaired amyloid-β peptide (Aβ) clearance from the brain caused by various factors including brain ischemia and hypoxia. Our studies in rats subjected to prenatal hypoxia (PH) suggest that a single episode of severe hypoxia in the critical period of brain development (E14, 7% O_2, 3 h) results in alteration of Aβ metabolism, impaired synaptic plasticity and cognitive deficit in postnatal life. Apart from increased amyloid precursor protein (APP) expression in rat brain caused by PH we also observed activation of caspase-3 and reduced levels of a major amyloid-degrading enzyme, neprilysin (NEP), correlating with decreased levels of a transcriptional factor AICD (C-terminal fragment of APP produced alongside Aβ). Manipulating AICD levels in HP rat brain by injections of the histone deacetylase inhibitor, sodium valproate, or with the caspase inhibitor Ac-DEVD-CHO in postnatal life resulted in restoration of NEP activity, the number of synaptic spines in the cortex and hippocampus, and improved cognitive functions. PH hypoxia also resulted in significant changes in the levels of a transport protein transthyretin (TTR) in the choroid plexus and other brain regions. Since both NEP and TTR play an important role in Aβ clearance, alterations of their expression might lead to disruption of Aβ homeostasis leading to impaired brain functions. Understanding mechanisms of amyloid clearance in the brain is of particular importance for designing strategies for prevention of neurodegeneration caused by Aβ accumulation. Supported by RFBR (16-04-00694), ARUK and MRC (UK).

Poster 272 – Monday 5th December

ALPHA-9 NICOTINIC ACETYLCHOLINE RECEPTOR MEDIATES HYPOTHERMIC RESPONSES ELICITED BY PROVOCATIVE MOTION IN MICE.

Eugene Nalivaiko[1], Longlong Tu[2], Lauren Poppi[1], John Rudd[2], Alan Brichta[1]

[1]University of Newcastle, [2]Chinese University of Hong Kong

Hypothermic responses accompany motion sickness in humans and could be elicited by provocative motion in rats. Our aim was to test whether similar responses are present in mice, and do determine potential role in these responses of efferent cholinergic vestibular innervation. To this end, we used knockout (KO) mice lacking ?9 cholinoreceptor subunit predominantly expressed in the vestibular hair cells and CBA strain as a wild-type (WT) controls. In WT mice, circular horizontal motion (1 Hz, 4 cm radius, 20 min) caused rapid and dramatic falls in core body temperature and surface head temperature associated with transient rise in the tail temperature; these responses were substantially attenuated in KO mice; changes were (WT *vs.* KO): for the core body temperature -5.2±0.3 *vs*. -2.9±0.3ºC; $p < 0.01$; for the head skin temperature -3.3±0.2 vs. -1.7±0.2ºC; $p < 0.01$; for the tail skin temperature +3.9±1.1 *vs* +1.1±1.2ºC, $p < 0.01$. There was a close correlation in the time course of cooling the body and the surface of the head. We conclude: i) that the integrity of cholinergic efferent vestibular system is essential for the full expression of motion-induced hypothermia in mice, and that the role of this system is likely facilitatory; ii) that in mice, motion-induced body cooling is mediated via increased heat low through vasodilated tail vasculature and (likely) via reduced thermogenesis. Our results support the idea that hypothermia is a biological correlate of nausea-like state in animals.

Poster 273 – Tuesday 6th December

TWO TYPES OF MELANOPSIN-EXPRESSING GANGLION CELLS IN THE HUMAN RETINA

Ms Subha Nasir Ahmad[1], Dr Sammy C Lee[1,2], Dr Paul R Martin[1,2,3], Dr Ulrike Grunert[1,2,3]

[1]Department of Ophthalmology and Save Sight Institute, The University of Sydney, [2]Australian Research Council Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]School of Medical Sciences, The University of Sydney

Melanopsin is a photopigment expressed in a subset of intrinsically photosensitive ganglion cells in the mammalian retina. Melanopsin-expressing cells are involved in non-image forming visual processes such as the pupillary light reflex and circadian entrainment. Recent research on the anatomical and physiological properties of melanopsin cells has mainly been performed in animal models. The present study characterizes melanopsin cells in the human retina by studying their morphology, distribution and synaptic connectivity. *Post mortem* human eyes (n= 3) were obtained from the Lions NSW Eye Bank at Sydney Eye Hospital with ethical approval. Eyes were fixed in 2% paraformaldehyde, the retinas dissected and processed with antibodies against melanopsin. The synaptic input to melanopsin cells was determined with antibodies against the postsynaptic density protein PSD95. Two distinct populations of melanopsin-expressing cells were identified based on their dendritic stratification in either the outer or the inner part of the inner plexiform layer. In addition some bistratified cells were seen. The dendrites of outer- and inner-stratifying cells cover the retina with highly overlapping dendritic arbors but the bistratified cells are part of the inner stratifying population. Outer-stratifying cells make up about 60 % of the melanopsin-expressing cells. The dendritic field size of melanopsin-expressing cells ranges from 250 µm (near the fovea) to 1000 µm in peripheral retina. For both inner and outer types the density decreases with the distance from the fovea. Both the outer and inner-stratifying dendrites showed colocalized PSD95 immunoreactive puncta suggesting that they receive synaptic input from bipolar cells.

Poster 274 – Monday 5th December

BURKHOLDERIA PSEUDOMALLEI RAPIDLY INFECTS THE BRAINSTEM AND SPINAL CORD VIA TRIGEMINAL NERVE AFTER INTRANASAL INOCULATION

Lynn Nazareth[1,2], Dr James St John[1,5], Heidi Walkden[1,2], Dr Kenneth Beagley[3], Dr Glenn Ulett[4,5], Dr Michael Batzloff[5], Dr Ifor Beacham[5], Dr Jenny Ekberg[1,2,3]

[1]Eskitis Institute for Drug Discovery, Griffith University, [2]Faculty of Health Sciences and Medicine, Bond University, [3]Institute for Health and Biomedical Innovation, Queensland University of Technology,, [4]School of Medical Science, Griffith University, [5]Institute for Glycomics, Griffith University

Melioidosis, a deadly disease caused by *Burkholderia.pseudomallei* is endemic to Thailand and northern Australia (mortality rate as high as 40% and 20% respectively), with neurological melioidosis being more prevalent in patients in Australia. People suffering from this form of melioidosis have reported to have bacteria invading their brain stem which can spread to the spinal cord. However the mechanism of how the bacteria enters the CNS is not well understood. We have previously reported that *B.pseudomallei* infects the olfactory nerves and the trigeminal nerves and therefore hypothesized that this bacteria might use the trigeminal nerve to enter the CNS. After intranasal inoculation of *B.pseudomallei* in mice, it produced a low level infection of the nasal epithelium before invading the trigeminal nerve in small numbers. The bacteria crossed the astrocytic barrier at the merge of the trigeminal nerve with the CNS and entered the brain stem and further travelled 2000 µm into the spinal cord, all within 24 hours. To rule out the bacterium might have used a haematogenous route, we used a capsule deficient mutant bacteria, which does not survive in the blood stream, and found that it too entered the CNS using the trigeminal nerve. We also showed that the bacteria uses actin mediated motility to enter the olfactory epithelium as loss *bimA* protein significantly reduced the ability of the bacteria to invade olfactory tissue. Thus we have shown that *B. pseudomallei* rapidly invades the trigeminal nerve that innervates the nasal cavity to infect the brainstem and spinal cord.

Poster 275 – Tuesday 6th December

ISTRADEFYLLINE AND PRELADENANT AS ADJUVANT THERAPIES FOR PARKINSON'S DISEASE

Ahmed Negida[1], Zeinab Hassan[1], Hussien Ahmed[1], Abdallah El-sherbiny[1], Shady Azzam[2], Omnya Osama[3], Osama Hassan[1], Yasmein Ibrahim[1]

[1]School of Medicine, Zagazig University, [2]School of Medicine, Al Azhar University, [3]Kasr Alainy School of Medicine, Cairo University

Introduction
Recent studies showed that adenosine A2a receptors are involved in the pathogenesis of Parkinson's disease (PD). Multiple A2a receptor antagonists have been developed and their efficacy has been assessed in multiple clinical trials. The aim of this meta-analysis is to synthesize evidence about the efficacy of the adenosine receptor antagonists (Istradefylline and Preladenant) for patients with Parkinson's disease.
Methods
We searched PubMed through, November, 2015, using relevant keywords. Records were screened for randomized controlled trials assessing the efficacy of Istradefylline and Preladenant in comparison to placebo. Data were extracted to online data extraction sheet and were analyzed. Outcomes of (off time, on time without troublesome dyskinesia, on time with troublesome dyskinesia, UPDRS III, and UPDRS II) were pooled as mean difference or weighted mean difference between the two groups from baseline to endpoints. Statistical analyses were conducted by RevMan version 5.3 for windows and Open[Meta-analyst].
Results
Ten RCTs (Istradefylline: 7 RCTs, n=2231; and Preladenant: 3 RCTs, n=1507 patients) were pooled in the final analysis. The overall effect estimate favored Istradefylline than placebo in terms of: (1) daily time off (20 mg/day: WMD -0.621, 95% CI -1.064 to -0.178; 40 mg/day: WMD -0.801, 95% CI -1.221 to -0.381); (2) on time without troublesome dyskinesia (20 mg/day: WMD 0.747, 95% CI 0.313 to 1.180; 40 mg/day: WMD 0.856, 95 % CI 0.401 to 1.312); and (3) UPDRS III "on state" (20 mg/day: WMD -0.917, 95% CI -1.717 to -0.117; 40 mg/day: WMD -1.612; 95 % CI -2.491 to -0.734). However, the overall effect estimate did not favor Istradefylline over placebo in terms of: (1) on time with dyskinesia (20 mg/day: WMD 0.891, 95% CI -0.662 to 2.444; 40 mg/day: WMD 0.982, 95 % CI -0.083 to 2.048); (2) UPDRS II during off state (20 mg/day: WMD -0.488, 95% CI -1.130 to 0.155; 40 mg/day: WMD -0.519, 95 % CI -1.301 to 0.263); and (3) UPDRS II during on state (20 mg/day: WMD 0.094, 95% CI -0.545 to 0.734); 40 mg/day: WMD -0.189, 95 % CI -0.491 to 0.114). For the Preladenant, the overall effect size favored Preladenant than Placebo in terms of "daily time off" (WMD -0.303, 95% CI -0.543 to -0.064). However, in the subgroup analysis, this effect size was not significant for the 2 mg, 5 mg, and 10 mg doses compared to placebo. In terms of "ON time without troublesome dyskinesia", the overall effect size did not favor either of the two groups (WMD 0.239; 95% CI -0.032 to 0.509) and the effect size was not significant for the subgroups of 2 mg, 5 mg, and 10 mg doses in comparison to placebo.
Conclusion
Istradefylline could improve the motor functions during the "on state" and it was effective in reducing the "off time" without increasing the "on time with troublesome dyskinesia". Current evidence suggests that Preladenant can reduce the "off time". However, further randomized controlled trials on Preladenant are needed.

Poster 276 – Monday 5th December

INHIBITION STUDIES OF KYNURENINE AMINOTRANSFERASE-2: A PROMISING TARGET IN NEURODEGENERATIVE AND COGNITIVE DISORDERS

Mr Alireza Nematollahi[1], Mr Guanchen Sun[1], Mr Gayan Jayawickrama[1], Professor Jane Hanrahan[2], Dr William Church[1]

[1]Group in Biomolecular Structure and Informatics, Faculty of Pharmacy, University of Sydney, [2]Faculty of Pharmacy, University of Sydney

The kynurenine pathway (KP), the main metabolic route of tryptophan, produces both neurotoxic and neuroprotective metabolites, and any changes that occur to their normal physiological balance can cause alterations in neuroactivity; as a consequence, it is believed KP metabolites are involved in a number of neurodegenerative and cognitive diseases.
Kynurenine aminotransferase (KAT) and kynurenine 3-monooxygenase have the most significant roles in regulating the KP. The elevated levels of KYNA (mainly produced by KAT-2) could be a trigger for cognitive disorders such as schizophrenia. Therefore, maintaining KP balance by regulating levels of KYNA directly through the inhibition of KAT-2 becomes a contemplatable therapeutic goal.
In our drug discovery approach, we performed studies on KAT-2 focusing on developing inhibitors. In our approach we have recently profiled our current lead, NS-1502, examined its inhibition potency and its probable mechanism of action using our HPLC-based assay under several reaction conditions; also we have determined its binding affinity to the human KAT-2 by surface plasmon resonance. The results obtained suggest NS-1502 is a promising inhibitor acting in a reversible mechanism with inhibitory activity almost 10 times more potent than the known reversible KAT-2 inhibitor, (S)-ESBA. Also we present the crystal structure of human KAT-2 in complex with NS-1502 at a resolution of 1.81 Å (PDB ID 5FT5), which will be valuable in the further design and optimization of reversible KAT-2 inhibitors, and may be significant in our approach to deriving a pharmaceutical intervention in order to overcome neurodegenerative and cognitive diseases.

Poster 277 – Tuesday 6th December

ARTEMIN, NEURTURIN AND GDNF RAPIDLY ACTIVATE AND SENSITIZE SENSORY NEURONS THAT INNERVATE BONE

Sara Nencini[1], Jason Ivanusic[1]

[1]University of Melbourne, Anatomy and Neuroscience

Our previous investigations have documented the properties of sensory neurons supplying bone that contribute to pain of bony origin. However, knowledge of the mechanisms that initiate and maintain inflammatory bone pain remains rudimentary. A number of studies have

recently suggested that Glial cell-line Derived Neurotrophic Factor (GDNF) Family Ligands (GFLs) are involved in the pathogenesis of inflammatory pain. Using an *in vivo* rat electrophysiological bone-nerve preparation, we tested the ability of artemin, neurturin and GDNF (GFLs) to activate bone afferent neurons in whole nerve recordings and sensitize single, mechanically activated bone afferent neurons. Application of artemin, neurturin and GDNF to the marrow cavity resulted in a rapid and transient increase in ongoing activity in whole-nerve recordings and sensitization (reduced threshold for activation and increased frequency of action potential discharge) of single mechanically activated neurons. Artemin sensitized more of the mechanically activated neurons (~70%) than did GDNF or neurturin (~30%). The time-course of the changes in ongoing activity (whole nerve recordings) and sensitivity (single mechanically activated neurons) was different for each of the GFLs. The results indicate that each of the GFLs are able to rapidly activate and sensitize bone afferent neurons, but they may have different roles in generating and/or maintaining bone pain. The changes we have observed are likely to be relevant to bony pathologies with a significant inflammatory component.

Poster 278 – Monday 5th December

DE NOVO MUTATIONS IN DENR DISRUPT NEURONAL DEVELOPMENT AND LINK CONGENITAL NEUROLOGICAL DISORDERS TO FAULTY MRNA TRANSLATION RE-INITIATION

Miss Linh Hoang Ngo[1,2], Doctor Matilda Haas[2], Miss Shan Shan Li[2], Ms Sibylle Schleich[3], Mr Zhengdong Qu[2], Doctor Hannah Kate Vanyai[1], Ms Hayley Daniella Cullen[1], Ms Aida Cardona Alberich[4], Doctor Ivan Eng Gladwyn-Ng[1,2], Doctor Alistair Pagnamenta[5], Professor Jenny Taylor[5], Doctor Helen Stewart[6], Doctor Usha Kini[6], Doctor Kent Duncan[4], Doctor Aurelio Teleman[3], Doctor David Anthony Keays[7], Associate Professor Julian Ik-Tsen Heng[1,2]

[1]Harry Perkins Institute Of Medical Research, [2]EMBL Australia, The Australian Regenerative Medicine Institute, Monash University, [3]German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, [4]Center for Molecular Neurobiology (ZMNH), University Medical Center Hamburg-Eppendorf, [5]National Institute for Health Research Biomedical Research Centre, Wellcome Trust Centre for Human Genetics, [6]Department of Clinical Genetics, Churchill Hospital, Old Road, [7]Institute of Molecular Pathology

Disruptions to neuronal mRNA translation are hypothesized to underlie human neurodevelopmental syndromes (Kelleher and Bear, 2008). Notably, the mRNA translation re-initiation factor DENR is a regulator of eukaryotic translation and cell growth (Schleich et al., 2014), but its mammalian functions are unknown. Here, we report that Denr promotes the migration of murine cerebral cortical neurons in vivo with its binding partner Mcts1, while perturbations to Denr impair the long-term positioning, dendritic arborisation and dendritic spine characteristics of postnatal projection neurons. We characterized *de novo* missense mutations in DENR (p.C37Y and p.P121L) detected in two unrelated human subjects diagnosed with brain developmental disorder to find that each variant impairs the function of DENR in mRNA translation re-initiation, and disrupts the migration and terminal branching of cortical neurons in different ways. Thus, our findings link human brain disorders to impaired mRNA translation re-initiation through perturbations in DENR function in neurons.

Poster 279 – Tuesday 6th December

ESTABLISHING THE RELATIONSHIP BETWEEN SECONDARY DNA STRUCTURES AND CENTRAL NERVOUS SYSTEM SCARRING.

Miss Michelle Nguyen[1], Mrs Priyanka Toshniwal[2,3], Mr Diwei Ho[2], Dr Helena Viola[4], Dr Mark Fear[3], Professor Fiona Wood[3], Professor K Swaminathan Iyer[2], Associate Professor Livia Hool[4], Professor Sarah Dunlop[1], Associate Professor Melinda Fitzgerald[1], Dr Nicole Smith[1,2]

[1]Experimental and Regenerative Neurosciences, School of Animal Biology, The University Of Western Australia, [2]School of Chemistry and Biochemistry, The University of Western Australia, [3]Burn Injury Research Unit, The University of Western Australia, [4]School of Anatomy, Physiology, and Human Biology, The University of Western Australia

Scar formation in the central nervous system (CNS) as a result of traumatic injury is both beneficial and detrimental, acting as a barrier to the spread of inflammation but also resulting in loss of calcium homeostasis and increased expression of growth inhibitory components, including chondroitin sulfate proteoglycans (CSPGs). Cations such as calcium have been shown to stabilise secondary DNA structures called G-quadruplexes (G4-DNA), which are over-represented in gene regulatory regions. We tested the hypothesis that G4-DNA formation is altered in CNS scarring due to an ionic imbalance. As the CNS scar consists of both a fibrotic layer, composed of invading fibroblasts, and a glial layer, predominantly consisting of reactive astrocytes, we evaluated alterations in G4-DNA formation using *in vitro* models of both fibrosis and astrogliosis. To mimic fibrosis, fibroblasts were stimulated with TGF-β over 4 days. A significant increase in collagen production was confirmed compared to unstimulated controls, by both qPCR and immunohistochemical analysis ($p \leq 0.05$). We further demonstrated an increase in intracellular calcium levels in stimulated fibroblasts using the fluorescent calcium indicator Fura-2AM and a significant increase in G4-DNA immunoreactivity, in comparison to unstimulated controls ($p \leq 0.05$). Using a G4-prediction algorithm, we have identified four G-quadruplexes in the Collagen 1 gene promoter, indicating that gene expression during fibrosis may be regulated by G-quadruplexes. Therefore, G-quadruplexes may have potential as therapeutic targets for the external control of gene expression, enabling silencing of inhibitory factor expression, and creation of a permissive environment for neural regeneration within the CNS scar.

Poster 280 – Monday 5th December

MILD HYPOXIA IMPROVES FUNCTIONAL OUTCOMES FOLLOWING ENDOTHELIN-1-INDUCED STROKE IN RATS

Nguyen HL[1], Fath T[2], Jones NM[1]

1. Department of Pharmacology, UNSW Australia, Sydney NSW 2052, Australia.
2. Neurodegeneration and Repair Unit, UNSW Australia, Sydney NSW 2052, Australia.

Stroke is the third most common cause of death and a major cause of neurological disability. Tissue plasminogen activator is currently the only approved treatment. However, due to its narrow therapeutic window, only 2-5% of patients are eligible for this therapy. In animal models of stroke, mild hypoxia (MH) after ischemia can reduce brain injury but effects on functional recovery and mechanisms of neuroprotection have not been determined.

Conscious male Sprague-Dawley rats were subjected to ischemia by perivascular microinjection of endothelin-1 onto the middle cerebra artery via a cannula, followed by MH (8% O_2, balanced N_2) or normoxia (room air) starting 24h post ischemia (1h/d for 5d). Behavioural tests were performed to assess neurological function. Immunoblotting was performed and brain tissue was collected for histological analysis.

Treatment with MH after stroke reduced the error rate in a foot fault test (9.5±3.0 vs 22.4±3.9%, $p<0.05$), decreased response time in the sensory hemineglect test (3.5±0.5 vs 28.9±10.62 sec, $p<0.05$) and reduced asymmetry scores in postural reflex test (0 vs 2 median scores, $p<0.05$). Improvement in these tests indicated better motor coordination, sensory perception and overall neurological function. Furthermore, MH reduced infarct size (5.9±1.1 vs 19.9±2.9 mm^3, $p<0.05$) and up-regulated levels of the transcription factor hypoxia inducible factor-1 (HIF-1) (0.32±0.02 vs 0.16±0.04, $p<0.05$) in the ipsilateral hemisphere.

Our data showed MH after stroke improved behavioural outcomes and reduced infarct size. This treatment assisted brain repair and it's likely that the transcription factor HIF-1 is an important regulator of hypoxia-induced functional recovery after stroke.

Poster 281 – Tuesday 6th December

TRPM8 CHANNELS ARE NOT INVOLVED IN THE EXCITATORY ACTION OF FREE RADICALS ON SENSORY NEURONS IN THE BLADDER.

Nicholas SJ, Brookes SJH, Spencer NJ, Zagorodnyuk VP.

Human Physiology and Centre for Neuroscience, FMST, School of Medicine, Flinders University, SA, Australia.

TRPM8 channels are expressed in both the urothelium and bladder sensory neurons. However, the role of TRPM8 channels in bladder function and pathophysiological changes are not well understood. We aim to establish the effect of TRPM8 agonist, icilin on different classes of bladder sensory neurons and to determine whether TRPM8 channels are involved in the action of free radicals on sensory neurons. Single unit extracellular recordings were made, in vitro, from fine nerve branches of pelvic afferent nerves innervating the guinea pig bladder. Icilin (5 μM) activated 43% (n=35) of capsaicin-sensitive high threshold afferents with mean firing rate of 0.35 ± 0.1Hz (n=12). Application of H_2O_2 induced concentration-dependent activation of high threshold afferents with an EC_{50} of 1.25 mM (95% CI =0.32-4.89 mM, n=9). Icilin (5 μM) also activated 29% (n= 38) of low threshold stretch-sensitive afferents with mean firing rate of 0.18 ± 0.05 Hz (n = 11). There was no correlation between the size of effect of H_2O_2 and icilin, when both drugs were applied on the same high threshold (n=14) or low threshold afferents (n=17). TRPM8 antagonist, M8-B (3 μM) did not affect the H_2O_2 (1 mM)-induced responses in high threshold afferents (-15 ± 16%, n=5, NS) although it significantly inhibited (by 78 ± 7%, n=5) the effect of icilin. The data indicate that TRPM8 channels are present in significant population of low threshold stretch-sensitive and high threshold bladder afferents. However, TRPM8 channels are not involved in the excitatory effect of H_2O_2 on bladder sensory neurons.

Poster 282 – Monday 5th December

BDNF HETEROZYGOUS MICE EXHIBIT REDUCED OLIGODENDROCYTE NUMBERS DURING POST-NATAL DEVELOPMENT

Madeline Nicholson[1], Rhiannon Wood[1], Simon Murray[1,2], Junhua Xiao[1,2]

[1]The University Of Melbourne, [2]The Florey Institute of Neuroscience and Mental Health Research

Myelination is a complex biological process and the extracellular factors responsible for inducing myelination in the central nervous system (CNS) remain incompletely understood. We have previously identified brain-derived neurotrophic factor (BDNF) as a key player in promoting CNS myelination during development, as BDNF heterozygous mice exhibit a severe hypomyelinating phenotype during early postnatal weeks. However, it remains controversial whether BDNF has an influence on oligodendroglial cell numbers and lineage progression *in vivo.*
To investigate this, BDNF heterozygous (HET) and wild-type (WT) littermate control mice were sacrificed at P9, P15 and P30. Both white and grey matter of the brain and lumbar spinal cord were analysed for the number of oligodendroglilal precursor cells (OPCs) and mature oligodendrocytes. We found significantly fewer oligodendrocytes in lumbar spinal cord grey matter (P=0.001) of BDNF HET mice at P15 compared to control WT mice. Whilst overall there were lower numbers of both oligodendroglial populations across tissues at P15 and P30 between genotypes, proportions of these cells remained not significant, indicating BDNF is likely influencing oligodendroglial proliferation and/or survival, but not differentiation in the brain and spinal cord *in vivo*.
Together, this study reveals an important role of BDNF signalling in regulating oligodendroglial populations during development, providing a new insight into understanding the cellular and molecular signalling pathways that influence CNS myelination.

Poster 283 – Tuesday 6th December

HOVERFLY VISION IN NATURALISTIC SURROUNDS

Dr Paloma Gonzalez-Bellido[3], Dr Shannon Olsson[2], **Dr Karin Nordstrom[1]**
[1]Centre for Neuroscience, Flinders University, [2]National Centre for Biological Sciences, Tata Institute of Fundamental Research, [3]PDN Department, University of Cambridge

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.

Poster 284 – Monday 5th December

MICE EXPRESSING MUTATIONS IN THE SYNAPTIC GENE NEUROLIGIN-3 SHOW ALTERED FLEXIBILITY IN A TOUCHSCREEN TEST OF RELATIONAL MEMORY

Ms Rebecca Norris[1], Professor Anthony Hannan[1], Dr Jess Nithianantharajah[1]

[1]Florey Institute Of Neuroscience And Mental Health

Neuroligins are a family of cell adhesion proteins that are crucial components of the post-synaptic density. Neuroligins form trans-synaptic complexes critical for synapse formation, maturation and function. Human mutations in neuroligin genes have been reported in brain disorders displaying cognitive symptoms. Specifically, mutations in neuroligin-3 (NL3) have been found in schizophrenia and autism spectrum disorders. To examine the impact of mutations in NL3 on cognition, we aimed to assess two genetic mouse models of NL3 dysfunction, the NL3 R451C knock-in (KI) and the NL3 knock-out, on a transitive inference task in the rodent touchscreen system that we recently developed. Transitive Inference (TI) measures relational memory and cognitive flexibility. TI involves training subjects on overlapping pairs of stimuli such that the higher member of each pair is rewarded, implying a linear hierarchy in reward contingency. Our results show that wild type C57BL/6 mice are robustly able to perform this visual touchscreen version of TI. Additionally, we observe that NL3 R451C KI mice show altered flexibility during task acquisition. Our findings suggest mutations in NL3 impact cognitive flexibility, highlighting an important role for NL3 in the regulation of cognitive functions relevant to human disorders.

Poster 285 – Tuesday 6th December

CHOLINERGIC AND PEPTIDERGIC INNERVATION OF OVARIAN TUMOURS

Oliveira S M R[1, 2, 3], Roselli S[1, 3], Hondermarck H[1, 3], Jobling P[1, 2]

2. *University of Newcastle, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, Callaghan, NSW 2308, Australia*
1. *Preclinical Neurobiology Research, The University of Newcastle, Australia*
2. *Hunter Cancer Research Alliance, The University of Newcastle, Australia*

The co-existence of cancer cells and nerves is increasingly reported in human tumours. Furthermore the presence of axons in tumours is associated with more aggressive cancers and poor patient prognosis (Jobling et al., Cancer Res., 75, P1777). Epithelial ovarian cancer is one of the most lethal gynaecological cancers worldwide. In a previous communication to the society we demonstrated axons containing the general nerve marker PGP 9.5 in ovarian tumours and that a proportion of these axons expressed immunoreactivity for tyrosine hydroxylase. In this study our aim was to further characterise the neuronal populations that invade ovarian tumours. Tissue microarrays (TMAs) from human ovarian biopsies were analysed by immunohistochemistry. Antibodies against the vesicular acetylcholine transporter (VAChT), substance P (SP) and neurokinin receptor 1 (NK1) were used. NK1 immunoreactivity (IR) was present in 8/10 tumours and two normal ovary sections. Substance P-IR was observed in 8/18 of normal ovarian sections and 48/202 tumours. SP –IR was observed in axons, presumed immune cells and neuroendocrine cells. VAChT-IR was observed in 170/202 ovarian tumours where it was expressed in axons, intrinsic neurons, cancer cells and other cells within the stroma. Our data suggest that cholinergic and peptidergic signalling is part of the ovarian tumour microenvironment and these pathways may offer novel therapeutic targets.

Poster 286 – Monday 5th December

IMAGESURF: AN IMAGEJ PLUGIN FOR ACCURATE AND UNBIASED QUANTITATION OF FLUORESCENCE IMAGES

Aidan O'Mara[1], Dr. Anna King[1], Professor James Vickers[1], Dr Matthew Kirkcaldie[1,2]

[1]Wicking Dementia And Education Centre, University of Tasmania, [2]School of Medicine, University of Tasmania

Quantitation of fluorescence images is integral to a wide range of neuroscience research. However, widely used threshold-based methods are sensitive to minor variation in staining and imaging. As an alternative, we have developed ImageSURF (Image Segmentation Using Random Forests), a free open-source ImageJ plugin. ImageSURF uses examples annotated by the user to derive rules to accurately distinguish specific features across large image sets, yielding consistent segmentations regardless of experimental conditions, and unbiased data without requiring experimenter blinding.

ImageSURF has been used for a range of confocal and epifluorescence images, including synapse and microglial markers. For the purposes of evaluation we compared it to optimised thresholding on confocal images of amyloid-beta plaques in a transgenic mouse model of Alzheimer's disease. Amyloid-beta pathology is difficult to quantify because plaque borders are typically diffuse, and slight variations in thresholds or image brightness can cause large variations in the measured plaque area. We trained ImageSURF using reference segmentations made by human raters, deriving generic rules which could reproduce these reference segmentations when applied to much larger image sets. In thresholding terms, this is equivalent to choosing a global threshold level on the basis of the reference set. In all cases, ImageSURF significantly ($p<0.05$) outperforms global thresholding, as measured by correlation between the reference segmentations and the outputs of ImageSURF and the best-performing threshold level. On this basis the criteria used to judge a small set of reference images can be applied across the entire image set, yielding an accurate, unbiased quantitation of pathology.

Poster 287 – Tuesday 6th December

CHRONIC STRESS EXACERBATES AMYLOID-BETA AND TAU PATHOLOGY AT THE THALAMIC SECONDARY NEURODEGENERATION FOLLOWING EXPERIMENTAL PHOTOTHROMBOTIC STROKE

Dr Lin Kooi Ong[1,2,3], Zidan Zhao[1,2], Murielle Kluge[1,2], Clifford TeBay[1], Giovanni Pietrogrande[1,2], Associate Professor Sarah J. Johnson[4], Professor Michael Nilsson[1,2,3], Associate Professor Frederick R. Walker[1,2,3]

[1]School of Biomedical Sciences and Pharmacy and the Priority Research Centre Stroke and Brain Injury, University of Newcastle, [2]Hunter Medical Research Institute, [3]NHMRC Centre of Research Excellence Stroke Rehabilitation and Brain Recovery, [4]School of Electrical Engineering and Computer Science, University of Newcastle

Stroke produces profound changes in the patient's life circumstances, such as motor and cognition impairments. The suddenness and severity of these deficits can mean that recovery from stroke can be very stressful. We have recently identified that chronic stress can exacerbate the severity of post-stroke thalamic secondary neurodegeneration. In this study, we investigated how exposure to chronic stress during the recovery period altered the composition of the neurotoxic protein, Amyloid-beta (Aβ) and the microtubule associated cytoskeletal protein, Tau within the thalamic secondary neurodegeneration sites. We utilised a focal model of cortical occlusion in adult C57B6 mice in combination with exposure to chronic restraint stress. Using immunohistochemistry we identified that stroke was associated with a significant deposition of Aβ, an effect that was exacerbated in stroke animals exposed to stress. Western blotting analysis indicated that stroke was associated with a significant enhancement of soluble Aβ oligomers and phosphorylation of Tau. Stroke animals exposed to chronic stress exhibited an additional increase in the expression of soluble Aβ oligomers and the phosphorylation of Tau. We further observed that Aβ accumulation and Tau hyperphosphorylation were associated with neuron loss. Given that Aβ and Tau pathology is recognised to correlate with accelerated cognitive decline, our results suggest that monitoring stress levels in patients recovering from stroke may merit consideration in the future.

Poster 288 – Monday 5th December

DOES THE PRIMARY CILIUM REGULATE OLIGODENDROCYTE PROGENITOR CELL PROLIFERATION?

Ms Megan O'Rourke[1], Dr Carlie Cullen[1], Dr Robert Gasperini[1,2], Dr Kaylene Young[1]

[1]Menzies Institute For Medical Research, [2]University of Tasmania

Oligodendrocyte precursor cells (OPCs) are a proliferative progenitor cell type that makes up ~5% of the cells in the adult human central nervous system (CNS). The primary cilium is an organelle that is known to regulate proliferation in a number of cell types, being associated with key proliferative signalling pathways, in particular the sonic hedgehog (Shh) signalling pathway. Despite the fact that oligodendrogenesis is highly regulated by Shh signalling, the presence of primary cilia on these cells has not been reported. To determine whether primary cilia could act as a signalling centre regulating OPC proliferation, we first aimed to determine whether primary cilia were expressed by cells of the oligodendrocyte lineage. By performing in situ hybridisation we readily detected the expression of *Ift81, Kif3a, patched* and *smoothened* mRNA in cultured mouse OPCs, indicating that genes important for primary cilium assembly and disassembly, and Shh signalling are expressed. By performing immunohistochemistry to detect Arl13b and γ-tubulin, we determined that 10% ± 6% of OPCs were ciliated *in vitro*, and 7.2% ± 0.2% of $SOX10^+$ oligodendrocyte-lineage cells were ciliated *in vivo*. Due to the proliferative nature of OPCs it is likely that their division is regulated by signalling at the primary cilium, and involves cilia assembly and disassembly. We expect that that ablation of the primary cilium *in vitro* by the removal of *Kif3a*, a gene important for assembly of the primary cilium cytoskeleton, we will substantially reduce OPC proliferation *in vitro* and *in vivo*.

Poster 289 – Tuesday 6th December

THE PHAGOCYTIC FUNCTION OF P2X7 RECEPTOR UNDER RESTING CONDITION IN THE CENTRAL NERVOUS SYSTEM

Ms Amber Ou[1], Professor James S Wiley[1], Dr Ben J Gu[1]
[1]*The Florey Institute Of Neuroscience And Mental Health*

P2X7 receptor is highly expressed on monocytes, macrophages and microglia. Activation of P2X7 by extracellular ATP induces large pore formation which leads to pro-inflammatory responses. These responses are implicated in pathogenesis of various neurological disorders particularly multiple sclerosis (MS). A second function of the P2X7 has been identified by our group. In the absence of ATP, P2X7 actively mediates phagocytosis. This function is particularly important in the central nervous system (CNS) because ATP is normally absent in cerebral spinal fluid. We studied P2X7 mediated pore formation using ethidium uptake assay and phagocytic function using fluorescent beads uptake assay. Peripheral blood monocytes from subjects were studied as a model for microglia of CNS. Our study shows that P2X7 antagonists (AF2789, A438079, A21060 10μM) potently inhibited P2X7 pore formation (>95% inhibition) but not phagocytosis. This antagonism was observed in monocytes harbouring various genetic variants including gain of function (348Thr) and loss of function alleles (568Gln). Furthermore, P2X7 mediated ethidium uptake was inhibited by presence of Na+ in the medium, while phagocytic function was the same in Na^+ or K^+ medium. We also found that divalent cations including Zn^{2+} and Cu^{2+} significantly inhibited phagocytosis. Moreover we studied a number of current MS treatments such as glatiramer acetate and vitamin D, and found they have diverse effects on P2X7 functions. These distinct features of P2X7 mediated phagocytosis suggest an important function of this receptor under resting conditions in the CNS, namely in the removal of apoptotic material and debris by cells expressing this receptor.

Poster 290 – Monday 5th December

ANDROGEN RECEPTOR GENE EXPRESSION IN THE DORSOLATERAL PREFRONTAL CORTEX IN PSYCHIATRIC DISORDERS

Miss Samantha Owens[1,2,3], Dr. Tertia Purves-Tyson[1,2,3], Dr. Maree Webster[4], Prof. Cynthia Shannon Weickert[1,2,3]

[1]*Schizophrenia Research Institute,* [2]*Schizophrenia Research Laboratory, Neuroscience Research Australia,* [3]*School of Psychiatry, University of New South Wales,* [4] *Laboratory of Brain Research, Stanley Medical Research Institute*

In schizophrenia, testosterone levels are inversely correlated with negative symptoms and low testosterone is linked to poorer cognitive performance, suggesting that androgens may contribute to brain pathophysiology. We investigated whether mRNAs of androgen-related molecules are altered in schizophrenia or bipolar disorder in post-mortem dorsolateral prefrontal cortex (DLPFC). Gene expression of 5α-reductase and androgen receptor (AR) in the NSW Brain Tissue Resource Centre cohort (TRC; 30 schizophrenia/7 schizoaffective/36 controls) and AR in the Stanley Medical Research Institute cohort (SMRI; 31 schizophrenia/31 bipolar disorder/31 controls) was measured using qPCR. In the TRC cohort, there were no changes in 5α-reductase mRNA ($F=0.39$; $df=72$; $p=0.68$), but there were changes in AR mRNA ($F=4.48$; $df=72$; $p=0.02$). AR mRNA was decreased in schizoaffective cases relative to schizophrenia cases ($p=0.005$) and there was a trend decrease relative to controls ($p=0.06$). There was also a trend increase in schizophrenia cases relative to controls ($p=0.08$). In the SMRI cohort, AR mRNA ($F=3.098$; $df=92$; $p=0.05$) was increased in bipolar disorder relative to controls ($p=0.05$) and unchanged in schizophrenia patients relative to controls. This suggests testosterone may be altered in patients with more affective symptoms and that cognitive and mood symptoms may be altered via androgenic mechanisms in some people with major mental illness. However, no large changes in AR mRNA in schizophrenia suggest that testosterone signalling may be more peripherally driven in these cases. Further investigations on gender differences and the effect of an AR genotype on brain androgen-related mRNAs are ongoing aspects of this work.

Poster 291 – Tuesday 6th December

BEHAVIOURAL AND PHYSIOLOGICAL TRANSGENERATIONAL RESPONSES TO PATERNAL EXERCISE

Dr Annabel Short[1], Ms Rachel Powell[1,2], Mr Shlomo Yeshurun[1], Dr Victoria Perreau[1], Dr Andrew Fox[1], Prof Moira O'Bryan[3], Dr Jee-Hyun Kim[1], Prof Anthony Hannan[1,2], **Dr Terence Pang**[1,2]

[1]*Florey Institute Of Neuroscience And Mental Health,* [2]*Department of Anatomy and Neuroscience,* [3]*Department of Anatomy and Developmental Biology*

We have previously provided evidence of a transgenerational response to chronic paternal mild stress which results in altered anxiety and depression-related behaviours of F1 and F2 generations (Short et al., Transl Psychiatry 2016). Here, we investigated if paternal exercise exerts a transgenerational effect by modifying anxiety and depression-related behaviours of offspring. Male C57Bl/6 mice were provided 4 weeks of voluntary wheel-running prior to paired-matings with naïve females. Male and female offspring underwent behavioural testing at PND15 and 8 weeks of age. We found a limited influence of paternal running on the elevated plus maze, light-dark box and forced-swim test. There was no significant impact on fear conditioning and extinction at early and adult ages. However, renewal of fear memory by male offspring was significantly altered in association with paternal running. Paternal stress altered the small RNA expression profile of sperm and we have found a similar effect following wheel-running using small RNA sequencing followed by validation in independent samples. Thus, besides conferring stress resilience in individuals, exercise could also influence the behavioural response to stress in the next generation via the male germ line.

Poster 292 – Monday 5th December

SATB2 AND CTIP2 EXPRESSION IN THE MARSUPIAL ISOCORTEX REVEALS A REGULATORY NETWORK THAT PREDATES EVOLUTION OF THE CORPUS CALLOSUM

Annalisa Paolino[1], Rodrigo Suárez[1], Peter Kozulin[1], Laura Morcom[1], Laura Fenlon[1], Linda Richards[1,2]
[1]The University of Queensland, Queensland Brain Institute, [2]The University of Queensland, School of Biomedical Sciences

Precise formation of brain connections is crucial for normal cognitive and sensory-motor functions. While the telencephalon of placental mammals, including humans, has three interhemispheric tracts, monotremes and marsupials only have two, as they lack a corpus callosum. Previous studies in mice show that corpus callosum development requires SATB2 expression whereby, when *Satb2* is knocked-out, callosal axons fail to form a corpus callosum and instead cross the midline via the anterior commissure. This phenotype resembles brain connectivity of montremes and marsupials, suggesting that *Satb2* may be mechanistically linked to callosal evolution. To investigate this, we analysed expression and function of transcription factors in the marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*, Dasyuridae). By performing retrograde neuronal tracing and *in-pouch* electroporation experiments coupled with immunofluorescence, we found that isocortical neurons projecting to the contralateral hemisphere via the anterior commissure are located in layers 2/3 and 5 and express SATB2 but not CTIP2, a marker of corticofugal neurons. Birthdate, layer distribution and molecular profile of commissural neurons were comparable to placental mammals, despite the strikingly different route taken by interhemispheric axons in both lineages. Finally, similar to mouse, corticofugal neurons located in layer 5 coexpress SATB2 and CTIP2, suggesting that the genetic regulatory networks of neuron projection fate arose before the origin of the corpus callosum.

Poster 293 – Tuesday 6th December

THE INTERACTION OF CALCIUM SIGNALING AND THE CYTOSKELETON IN NAVIGATING GROWTH CONES

Miss Macarena Pavez[1], Mr. Adrian Thompson[1], Associate Professor Lisa Foa[1], Dr. Robert Gasperini[1]

[1]University Of Tasmania

The precise connectivity that underlies neural circuitry in the central nervous system is regulated by axon guidance. A highly specialized sensory structure at the tip of extending axons, the growth cone, regulates axon guidance by responding to extrinsic guidance cues in the embryonic environment. Regulation of growth cone motility and axon guidance are thought to underlie a range of neurological disorders such as autism and schizophrenia due to demonstrated defects in neuronal targeting and connectivity. Spatially-restricted calcium signals regulate growth cone responses to guidance cues, but the mechanisms that regulate spatiotemporal calcium signalling are not clear. We hypothesize that STIM1, which is embedded in the membrane of the endoplasmic reticulum (ER), a major source of cytosolic calcium, is necessary for growth cone motility and guidance. Data obtained using high resolution imaging show that STIM1 interacts with and regulates the assembly and organization of microtubules during growth cone motility through an interaction with microtubule-binding protein EB-1/3. Reduction of STIM1 expression disrupted microtubule polymerisation ($p<0.0005$) and the asymmetric distribution of microtubules in growth cones turning towards BDNF ($p<0.0005$) or away from Sema3a ($p<0.05$). These data support the hypothesis that STIM1 is critical for the spatial localisation of calcium signals at the growth cone where the asymmetric regulation of ER translocation to the motile side of growth cones, mediated by STIM1- EB-1/3, controls the spatiotemporal release of instructive calcium signals. Our work significantly builds on the current understanding of how calcium regulates the growth cone navigation during early neuronal connectivity.

Poster 294 – Monday 5th December

DETECTING AND TREATING INFLAMMATORY BOWEL DISEASE USING NEUROMODULATION TECHNOLOGY

Dr Sophie C Payne[1,2], Professor Robert K Shepherd[1,2], Professor John B Furness[3], Associate Professor James B Fallon[1,2]

[1]Bionics Institute, [2]Medical Bionics Department, the University of Melbourne, [3]Florey Institute of Neuroscience and Mental Health

Inflammatory bowel disease (IBD) is a debilitating life-long autoimmune condition that is poorly controlled by pharmacological treatments. A number of studies demonstrate that neuromodulation of the vagus nerve reduces the symptoms of IBD (Sun et al., 2013). Here we aim to develop a closed-loop bionic device that will regulate electrical stimulation of the vagus nerve based on measurements taken from a key biomarker of IBD. First we developed an intestinal interface that provides objective real-time measurement of gut inflammation by measuring characteristic inflammatory-induced changes in transmucosal gut permeability. Using a rat model of IBD (intraluminal injection of 1mg/ml 2,4,6-Trinitrobenzene sulphonic acid) our intestinal interface detected significant increases (n=6; $P < 0.05$) in transmucosal permeability at 3 hours following inflammatory stimulus. No permeability changes were seen in control tissue ($P \geq 0.05$). Histological analysis of inflamed tissue taken from these permeability experiments showed significant increases in neutrophil, eosinophil and T-cell populations (n=6; $P < 0.05$), thereby demonstrating a correlation between inflammation and gut permeability. Following these acute studies, the intestinal interface was chronically implanted to assess transmucosal permeability in naive awake rats (n=3), and demonstrated that background variations in permeability were less than that induced during inflammation. These data provide confidence that this technique provides a safe and effective biomarker for chronic monitoring of IBD. The next phase of this research is to develop a safe peripheral nerve array to effectively stimulate the c-fibres of the abdominal vagus nerve. We anticipate this device will allow for patient-specific control over IBD.

Poster 295 – Tuesday 6th December

A NEW TECHNIQUE TO MEASURE DOPAMINE RELEASE IN THE MAMMALIAN RETINA

PhD Victor Perez Fernandez[1], Doctor David Harman[1], Professor John Morley[1], Doctor Morven Cameron[1]

[1]Western Sydney University. School of Medicine.

Adaptation of the retina to the presenting light conditions relies considerably on modulation of retinal pathways by dopamine (DA). DA is released in response to light by a unique set of neurons: dopaminergic amacrine cells (DACs). Cones, rods and intrinsically photoresponsive retinal ganglion cells (ipRGCs) have all been shown to input, in some way, to the DACs. However, the precise circuitry by which these signals reach the DACs is not completely known. We have previously shown that ipRGCs do not cause global DA release in mice lacking rods and cones, despite the existence of a well-characterized electrophysiological input. To probe this discrepancy in more detail we have optimised a novel method for DA quantification based on organic solvents, ultra-high performance liquid chromatography (UPLC) and mass spectrometry (MS). Compared with traditional methods based on inorganic solvents, high performance liquid chromatography (HPLC) and coulometric quantification, this new protocol exhibits significantly improved sensitivity and selectivity. We show that this method consistently reports a large increase in DOPAC/DA ratio, a reliable measure of DA release, when the retina is exposed to bright white light (1000 lux) in wild-type mice. We applied these methods to the retinal degenerate *rd/rd* mouse that lacks rods and cones, and the $Gnat2^{A517G}$ mouse which lacks functional cone transduction.

Poster 296 – Monday 5th December

VITAMIN D, VDR AND THE DIFFERENTIATION OF DOPAMINERGIC CELLS

Dr. Renata Pertile[1], Dr. Xiaoying Cui[1], Dr. Darryl Eyles[1,2]

[1]Queensland Brain Institute, [2]Queensland Centre for Mental Health Research

Vitamin D regulates multiple factors involved in the ontogeny of dopaminergic systems. It has been shown that in neonatal rats maternally deprived of vitamin D, dopamine (DA) turnover is decreased and the levels of the enzyme catechol-o-methyl transferase (COMT) is reduced. In order to examine the mechanisms by which the active vitamin D hormone, $1,25(OH)_2D_3$, via its receptor vitamin D receptor (VDR), affects DA production and turnover, we have over-expressed the VDR in neuroblastoma SH-SY5Y cells and analysed DA production by HPLC and expression of dopaminergic-associated genes by QPCR and Western Blot. Chromatin immunoprecipitation was used to analyse the binding of VDR in COMT promoter. Our results show that VDR overexpression increases DA neuron differentiation by increasing tyrosine hydroxylase expression and DA production. In the VDR-overexpressing cells, $1,25(OH)_2D_3$ further increased the levels of the DA-metabolites 3-MT and HVA and elevated *COMT* gene expression. Chromatin immunoprecipitation revealed that $1,25(OH)_2D_3$ increased VDR binding in 3 regions of the *COMT* promoter, suggesting direct regulation. The expression of other genes involved in the maturation of DA neurons such as *VMAT2*, *MAOA*, *DRD2* was also significantly altered in these cells. *NEUROG2,* a marker of immature DA neurons, also had its expression decreased. These results show VDR and $1,25(OH)_2D_3$ are directly involved in the regulation of dopaminergic-associated gene expression in neuronal cells and this model is a useful tool for identifying the role of $1,25(OH)_2D_3$ in DA neuronal development and maturation.

Poster 297 – Tuesday 6th December

CHARACTERISATION OF ONE CLASS OF SENSORY NEURONS IN SKELETAL MUSCLE IN VITRO

Mrs Rochelle Peterson[1], Dr Christine Barry[2], Professor Simon Brookes[1]

[1]Discipline of Human Physiology, FMST, School of Medicine, Flinders University, Adelaide, SA, [2]Discipline of Anatomy and Histology, FMST, School of Medicine, Flinders University, Adelaide, SA

Group III and group IV afferents in skeletal muscle are small diameter sensory nerves involved in sensation, nociception and reflex control of muscle. To date, it has not been possible to correlate morphological and physiological properties of small diameter muscle afferents. We investigated *in vitro,* sensory neurons innervating skeletal abdominal muscle in C57/Bl6 mice. Anterograde labelling was combined with immunohistochemistry to characterise axons by morphology, location and neurochemical content. Six classes were distinguished; motor axons (terminating in motor end plates), muscle spindles, vascular afferent axons, connective tissue afferent axons, intramuscular afferent axons and axons-of-passage. Extracellular recordings were made from subcostal nerve trunks innervating abdominal muscles. One class of afferent, selected for this study, comprised mechano-sensitive units with conduction velocities from 15-30m/s (7 units, n = 6). They increased firing in response to punctate compression and had saturating responses to graded stretch. They were not activated by capsaicin (10 µM) and did not fire spontaneously (20 units, n=23). Each of these mechanosensitive units had a single receptive field averageing $0.38\pm0.18mm^2$ (6 fields, 6 units; n = 6). Recorded nerve trunks were anterogradely filled with biotinamide to reveal their axons. In 6 cases, a single axon was identified which branched in the deep connective tissue; none of these axons was CGRP-immunoreactive. Here we have characterised a class of sensory axons in skeletal muscle, correlating their morphology and function, using techniques that have previously been used in viscera.

Poster 298 – Monday 5th December

ELEVATED DOPAMINE RELEASE IN THE DORSAL STRIATUM: A NEW ANIMAL MODEL OF SCHIZOPHRENIA

Alice Petty[1], Dr Xiaoying Cui[1], Professor Darryl Eyles[1,2]

[1]The Queensland Brain Institute, [2]Queensland Centre for Mental Health Research

Increased dopamine synthesis and release in the dorsal striatum is one of the strongest pathophysiological findings in patients with schizophrenia. Furthermore, this abnormality is already developing in the 'prodromal' period. Greater understanding of the prodrome is critical to potentially intervene in the transition to schizophrenia. Therefore we are developing an animal model of the prodrome. We used an AAV vector to insert TH and GCH1 bilaterally into the substantia nigra pars compacta (SNpc) of adolescent (P35) rats. TH and GCH1 are rate-limiting enzymes in the synthesis of dopamine, and the SNpc projects to the dorsal striatum. Six weeks after injections, animals were tested for amphetamine-induced locomotion (0.6mg/kg AMPH). Brain tissue was collected for neurochemical analyses. We found increased TH protein staining intensity in the SNpc, and an increase in human TH mRNA in the dorsal striatum, but not the NAc or PFC. There was a significant increase in the DA metabolite HVA in the dorsal striatum, and indications of decreased COMT levels in the dorsal striatum and PFC. Animals injected with the active virus showed significantly increased hyper-locomotion following the amphetamine treatment. These results indicate that the viral injection has been selective to the SNpc, and that the virus has successfully increased TH enzyme levels in the dorsal striatum. The changes in HVA might suggest increased DA turnover in the treated animals. Crucially, treated animals demonstrated hypersensitivity to amphetamine. This strongly suggests that this novel animal model is replicating phenotypes relevant to schizophrenia.

Poster 299 – Tuesday 6th December

EPIGENETIC DYSREGULATION OF CRITICAL GENE REGULATORY ELEMENTS IN AD MICE

Mr Andrew Phipps[1], Prof James Vickers[1], Dr Timothy Mercer[2], Dr Adele Woodhouse[1], Dr Phillippa Taberlay[3,4,5]

[1]Wicking Dementia Research And Education Centre, Faculty of Health, University of Tasmania, [2]Transcriptomics Research Laboratory, Genomics and Epigenetics Division, Garvan Institute of Medical Research, [3]School of Medicine, Faculty of Health, University of Tasmania, [4]Epigenetics Research Laboratory, Genomics and Epigenetics Division, Garvan Institute of Medical Research, [5]St. Vincent's Clinical School, Faculty of Medicine, University of New South Wales Australia

Introduction: Alzheimer's disease (AD) is a terminal progressive neurodegenerative disorder, yet the underlying cause of sporadic AD cases remains unknown. Epigenetic alterations such as DNA methylation and post-translational histone modifications may contribute to the pathological pathways implicated in the onset and progression of sporadic AD. **Methods**: APP/PS1 AD mice closely recapitulate the pathology present in human early-AD cases, including beta-amyloid plaque deposition and plaque-associated synapse loss. APP/PS1 mice enable us to examine the earliest epigenetic changes that occur in AD. Nuclei from the forebrain of APP/PS1 mice and age-matched wild-type control mice (n=3 per genotype at 10 months of age) were purified and subject to chromatin immunoprecipitation and next-generation sequencing (ChIP-seq) with antibodies detecting H3K4me3, H3K27me3, H3K4me1, and H3K27Ac. **Results**: Enhancer (H3K4me1, H3K27Ac) and promoter (H3K4me3, H3K27me3) marks are lost from regulatory regions in APP/PS1 mice compared to age-matched controls ($p<0.05$). Specifically, both enhancer and promoter marks were lost from key risk factor genes for sporadic AD (*PICALM, BIN1*; $p<0.05$) or at known differentially expressed genes in sporadic human cases and transgenic AD mice (*PICALM, TBXA2R, F2RL2, SORBS3*; $p<0.05$). **Conclusions:** These data show that epigenetic reprogramming occurs in a mouse model of early AD, affecting both enhancers and promoters. Epigenetic dysregulation may be a key aspect of AD pathology.

Poster 300 – Monday 5th December

CORRELATION OF LOCAL FIELD POTENTIAL POWER IN LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX IN ANAESTHETISED MARMOSETS.

Dr Alexander NJ Pietersen[1,2,3], Dr Calvin D Eiber[1,2,3], Natalie Zeater[1,2,3], Dr Samuel G Solomon[3,4], Prof Paul R Martin[1,2,3]

[1]Billson Research Laboratory, Save Sight Institute, The University Of Sydney, [2]ARC Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]School of Medical Sciences, The University of Sydney, [4]Experimental Psychology, University College London

Purpose: We previously reported that some neurons in the intercalated (koniocellular, K) layers of the lateral geniculate nucleus (LGN) show high variability in spike rate in absence of visual stimulation. Spike rates of K cells are higher when low frequency power (delta) in the electroencephalogram (EEG) in primary visual cortex (V1) is lower (Cheong SK et. al., PNAS, 2011). Our purpose here is to find out if local field potentials in LGN also show this relationship.
Methods: Local field potential in LGN (NeuroNexus 16x2 electrode) and V1 (bipolar and 4x4 Utah array) was recorded in Sufentanil-anaesthetised marmosets (*Callithrix jacchus*, n=2). Animals looked at a uniform grey field near 50 Cd / m^2. Low pass filtered (250 Hz) local field potential was analysed using fast Fourier transform with a moving window (3 seconds long, 0.3 second step). Band power for delta, theta, alpha, beta and gamma frequencies in the LGN was calculated from power spectra at each electrode and correlated with V1 delta band power.
Results: No LGN frequency band showed any significant correlation with V1 delta band power in 7 recording from 2 animals. Average correlation coefficient was close to 0 for all LGN frequency bands analysed (delta -0.002?0.05, theta 0.05?0.06, alpha 0.04?0.05, beta 0.03?0.06, gamma -0.003?0.04).
Conclusion: We could not detect a local field potential analogue for brain state induced changes in LGN spike rate. This suggests that the cause of changes in maintained spike rate is very local and/or not synchronized across the LGN.

Poster 301 – Tuesday 6th December

ELECTRICAL PROPERTIES OF OLIGODENDROCYTE PROGENITOR CELLS WITH ALZHEIMER'S DISEASE ASSOCIATED MUTATIONS

Dr Kimberley Pitman[1], Solène Ferreira[1], Dr Laura Clarke[2,3], Dr Carlie Cullen[1], Prof David Attwell[2], Dr Kaylene Young[1]

[1]Menzies Institute for Medical Research, [2]University College London, [3]Stanford University

Patients with incipient, mild or severe Alzheimer's disease (AD) experience white matter atrophy that correlates with cognitive decline. Transgenic mice modelling aspects of AD pathology also display an early reduction in myelin specific protein expression and changes to myelin ultrastructure. Furthermore, cuprizone-induced demyelination of pre-symptomatic Aβ-transgenic mice, produces hippocampal memory deficits that are not observed in demyelinated wildtype mice or untreated transgenic controls, suggesting that demyelination may be a precipitating event for cognitive decline in AD. We therefore propose that oligodendrocyte loss occurs early in AD pathology, but is initially undetected due to the activity of oligodendrocyte progenitor cells (OPCs). To evaluate how OPCs respond in the early stages of AD-like pathology, we performed whole cell patch clamp analysis of OPCs in the hippocampus of mice that: 1) express a mutant form of human amyloid precursor protein (*PDGFB-APPSwInd*); 2) express a mutant form of human microtubule associated protein tau (*Prnp-MAPT^{P301S}*); 3) express both (AD), or 4) were their wildtype littermates. We found that genotype did not alter the resting membrane potential, membrane capacitance or membrane resistance of OPCs at postnatal day (P)30 or P100. We next determined whether AD-like pathology altered the ability of OPCs to respond to neurotransmitters. We bath applied agonists of AMPA/kainate (kainate, 100 μM) or GABA (GABA, 100 μM) receptors and measured the evoked currents. There was no difference in the way that OPCs responded to kainate, however at P100 OPCs from AD mice responded more robustly to GABA than OPCs in wildtype mice ($P<0.03$).

Poster 302 – Monday 5th December

ENHANCED HEPARIN BINDING AFFINITY OF APP96-110 RESULTS IN INCREASED NEUROPROTECTION FOLLOWING TRAUMATIC BRAIN INJURY

Ms Stephanie Plummer[1], Dr Emma Thornton[1], Dr Frances Corrigan[1], Professor Roberto Cappai[2], Associate Professor Corinna Van Den Heuvel[1]

[1]Discipline of Anatomy and Pathology, School of Medicine, The University of Adelaide, [2]Department of Pathology, The University of Melbourne

Following traumatic brain injury (TBI), neurological damage is serious and ongoing. Recently, the amyloid precursor protein (APP) derivative APP96-110 has shown encouraging neuroprotective activity following TBI, believed to be due to its heparin-binding properties. It is hypothesised that mutation of key amino acid residues responsible for these properties could enhance its binding ability and subsequent therapeutic efficacy. To determine this, key residues were mutated on wildtype APP96-110, and its heparin-binding affinity assessed via chromatography assay. Its efficacy was then assessed *in vivo* following moderate-severe diffuse impact-acceleration injury in rats. A single dose of either wildtype APP96-110 (0.05m/kg or 0.5mgkg) or mutated APP96-110 (0.05mg/kg, 0.25mg/kg, 0.1mgkg or 0.5mg/kg) was administered intravenously at 5 hours post-TBI. Rats were assessed daily for motor deficits using the rotarod, before brains were perfused fixed following 3 days. Following TBI, rats treated with wildtype APP96-110 demonstrated significant improvements in motor outcome over 3 days when compared to vehicle control rats. Treatment with 0.25mg/kg of mutated APP96-110 significantly improved motor performance post-TBI when compared to vehicle control rats, reaching a similar level as the 0.5mg/kg wildtype APP96-110 treatment. A similar trend was seen throughout, where lower doses of mutated APP96-110 showed improved motor outcome on a par with higher doses of wildtype APP96-110. Unexpectedly, the highest dose of mutated APP96-110 (0.5mg/kg) was unable to improve motor outcome, indicating a maximal therapeutic threshold. These results indicate that through enhancing the heparin-binding affinity of APP96-110, lower doses may be used to improve its neuroprotective efficacy *in vivo* post-TBI.

Poster 303 – Tuesday 6th December

DEGENERATION OF CHOLINERGIC SYNAPTIC TRANSMISSION IN TYPE II VESTIBULAR HAIR CELLS OF AGEING C57BL/6 MICE

Ms Lauren Poppi[1], Mr Mark Bigland[1], Prof. Robert Callister[1], Dr. Paivi Jordan[2], A/Prof. Joseph Chris Holt[2], Dr. Rebecca Lim[1], Prof. Alan Brichta[1], Dr. Doug Smith[1]

[1]The University Of Newcastle, [2]University of Rochester

The loss of cholinergic networks in particular has long been associated with ageing, however it is not yet known whether the age-related decline of peripheral vestibular function is related to its cholinergic efferent innervation. Using patch clamp electrophysiology, we recorded the postsynaptic responses of type II vestibular hair cells to 300μM acetylcholine in cristae of mice aged 3 weeks (juvenile), 3-6 months (adult), and 28-32 months (aged). Antagonists against nicotinic acetylcholine receptors containing alpha9 subunit (strychnine; 1μM) and small conductance calcium-activated potassium channels, SK, (apamin; 0.1μM) were used to characterise channel activation in response to ACh. In addition, using RT-PCR, the expression levels of acetylcholine receptor mRNA from homogenised cristae of juvenile, adult and aged mice were compared. The number and morphology of cholinergic terminals in the cristae of all three groups were also compared using immunofluorescence and confocal microscopy. Preliminary evidence shows the peak amplitude and duration of ACh evoked responses were reduced in the aged cohort compared to juvenile mice. This was attributed to a decrease in both alpha9 nicotinic receptor subunit

and SK channel conductances. Similarly, there was a significant (~50%) concomitant decrease in alpha9 nicotinic receptor subunit mRNA in the cristae of aged mice compared to young adult controls. Age-related vestibular decline is likely to involve the peripheral cholinergic efferent system to some extent, as our data show significant decreases in expression and function of alpha9 subunit nicotinic receptors with increasing age.

Poster 304 – Monday 5th December

ANTI-EPILEPTOGENIC EFFECTS OF A SELECTIVE T-TYPE CA2+ CHANNEL ANTAGONIST, Z944, IN THE POST-STATUS EPILEPTICUS MODEL OF TEMPORAL LOBE EPILEPSY.

Dr Pablo Casillas-Espinosa[1], Emma Braine[1], Dr Sandy Shultz[1], Dr Nigel Jones[1], Prof Terry Snutch[2], Prof Terry O'Brien, **Dr Kim Powell[1]**

[1]The University Of Melbourne, The Department of Medicine, [2]Michael Smith Laboratories

Objective: Temporal lobe epilepsy (TLE) is the most common form of drug-resistant epilepsy. Current pharmacotherapy for TLE is symptomatic, suppressing seizures, but has no disease modifying effect on epileptogenesis. T-type Ca^{2+} channels have been implicated in the pathogenesis of TLE. In this study we evaluated the effects of Z944, a potent and selective T-type Ca^{2+} antagonist, on epileptogenesis in the post-status epilepticus (post-SE) model of TLE.
Methods: Wistar rats underwent implantation of EEG electrodes and kainic acid-induced SE for 4 hours. Treatment groups were: post-SE + Z944 (60mg/kg/day); post-SE + levetiracetam (200mg/kg/day); post-SE + vehicle; sham + vehicle or sham + Z944 (60mg/kg/day). Treatments were delivered by continuous subcutaneous infusion for four weeks followed by two weeks of continuous video-EEG monitoring.
Results: Post-SE + vehicle animals had the highest average number of seizures/day (0.77±0.09). Treatment with Z944 greatly reduced the number of seizures/day (0.017±0.012) which was significantly different compared to vehicle and levetiracetam treated animals (p<0.0001). Only two of the eight post-SE + Z944 animals had one seizure in the two week recording period, whereas all of the animals in the other post-SE groups had several seizures.
Conclusion: Treatment with Z944 has a powerful anti-epileptogenic effect in the post-SE model of TLE. This indicates that pharmacologically targeting T-type Ca^{2+} channels may be an effective disease-modifying treatment for TLE. Z944 has been found to have a favourable safety profile in early phase clinical trials for pain facilitating the translation of the results of this preclinical study into a clinical anti-epileptogenesis trial.

Poster 305 – Tuesday 6th December

VENTRAL PALLIDUM OUTPUT PATHWAYS IN CONTEXT-INDUCED REINSTATEMENT OF ALCOHOL SEEKING.

Dr Asheeta Prasad[1], Professor Gavan McNally[1]

[1]University Of New South Wales, Sydney, 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), lateral hypothalamus, 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 and chemogenetic approaches. First, using dual retrograde tracing, we show that VP neurons projecting to either VTA or STN are recruited during context-induced reinstatement of extinguished alcohol seeking in rats. Then, using chemogenetics, we show modulation of context-induced reinstatement and reacquisition of alcohol seeking via DREADD excitation or inhibition of the VP. To determine the causal roles of VP VTA and VP STN pathways in context-induced reinstatement and reacquisition we used a chemogenetic disconnection approach and show that silencing either the VP VTA or VP STN pathways is sufficient to reduce both reinstatement and reacquisition of alcohol seeking. Moreover, these disconnections also each reduced responding and motivation during a progressive ratio test but had no effect on locomotor activity. Taken together, these results show that multiple ventral pallidal output pathways contribute to relapse to alcohol seeking.

Poster 306 – Monday 5th December

ESTABLISHING THE NATURAL COURSE OF PERIPHERAL NEUROPATHY IN A RAT MODEL OF EARLY STAGE TYPE 2 DIABETES.

Dr Dino Premilovac[1], Mr Oleksandr Dorohokuplia[1], Mr Abraham Daniel[1], Dr Robert Gasperini[1], Professor Bruce Taylor[2], Dr Michelle Keske[2], Associate Professor Lisa Foa[1]

[1]School of Medicine, Faculty of Health, University Of Tasmania, [2]Menzies Institute for Medical Research, University Of Tasmania

Peripheral neuropathy affects more than 50% of type 2 diabetics (T2D) and manifests as abnormal sensitivity to sensory stimuli such as touch or heat. The majority of investigators have used the high fat diet (HFD) plus streptozotocin (STZ) injected rodent to investigate diabetic neuropathy. However, this model closely resembles type 1 diabetes, rather than T2D which accounts for the majority of human diabetic neuropathy. Using osmotic mini-pumps to delivery STZ, we have developed a new model of early stage T2D. The aim of the present study was to establish the time-course of peripheral neuropathy in this model. Male Sprague Dawley rats were maintained on control or HFD for 20 weeks. Following three weeks of HFD, animals were divided into (i) HFD only; (ii) HFD+vehicle (citrate buffered saline, pH 4.4); and (iii) HFD+STZ (120mg/kg). Osmotic mini-pumps were implanted subcutaneously and delivered vehicle and STZ solutions over 14 days. Development of glucose intolerance was determined by regular glucose tolerance testing. Voluntarily hind-paw withdrawal to tactile

(plantar aesthesiometer) and thermal (Hargraves test) stimuli were assessed weekly over 20-weeks. Osmotic mini-pump delivered STZ significantly worsened glucose tolerance relative to HFD only and vehicle groups and this response was maintained for the duration of the study. Hypersensitivity to touch was evident in T2D animals 5-6 weeks post-STZ and remained significantly different compared with vehicle treated animals. In contrast, no differences in thermal sensitivity were detected at any time-point. Our results highlight a temporal dissociation between peripheral touch and thermal sensitivity during early stages of T2D.

Poster 307 – Tuesday 6th December

CORTICAL RESPONSES TO STIMULUS TRANSITIONS ON SHORT AND LONG TIMESCALES REFLECT NEURONAL INTEGRATION AND ADAPTATION

Mr Brian Oakley[1], Dr Elizabeth Zavitz[1], Professor Marcello Rosa[1], **Dr Nicholas Price[1]**

[1]Monash University

Studies of the visual system commonly assess neuronal responses to sustained, unchanging, stimuli. However, accurately perceiving a dynamic world demands a visual system that can both temporally integrate and segregrate stimuli over time. To understand these competing processes, we examined how motion-sensitive neurons encode dynamic changes, or transitions, in motion direction.
We recorded extracellular activity from 164 single- and multi-units in the middle temporal area of 3 sufentanil- and N_2O-anaesthetised marmosets (*Callithrix jacchus*). The receptive fields of all neurons were stimulated with a continually moving random-dot field. In different stimulus blocks, the motion direction abruptly changed either every 33 ms or every 500 ms. We examined neuronal responses around the time of these transitions, and their dependence on the magnitude and frequency of the direction changes.
When transitions occurred every 33 ms, two distinct response patterns were evident. For small changes in direction (<90°), the neuronal population response smoothly transitioned from representing the pre- to the post-transition direction. For large changes in direction, neurons effectively stopped representing the first stimulus before responding to the second stimulus direction. Similar results were observed when transitions occurred every 500 ms, however, response amplitudes evoked by the second stimulus were much smaller when the direction change was small.
Our results are explained by a model in which adaptation over long time scales (~500 ms) causes a reduction in responsiveness, without affecting response integration. Critically, on short time scales, neurons non-linearly integrate over a wide range of directions, and over time periods >33 ms.

Poster 308 – Monday 5th December

HYPERACTIVITY AND DYSINHIBITION-LIKE BEHAVIOR IN A P301S TAU-TRANSGENIC MOUSE MODEL OF FRONTOTEMPORAL DEMENTIA

Ms Magdalena Przybyla[1], Dr. Claire Helen Stevens[1], Ms. Julia van der Hoven[1], Dr. Anne Harasta[1], Dr. Mian Bi[1], Dr. Arne Ittner[1], Dr. Annika van Hummel[1], Prof. John R Hodges[2], Prof. Olivier Piguet[2,3], Prof. Tim Karl[2,4], Prof. Michael Kassiou[5], Prof. Gary Housley[6], Dr. Yazi D Ke[7], Prof. Lars Ittner[1,2,8], Dr. Janet van Eersel[1]

[1]Dementia Research Unit, UNSW, [2]Neuroscience Research Australia, [3]ARC Centre of Excellence in Cognition and its Disorders, [4]School of Medicine, Western Sydney University, Campbelltown, [5]School of Chemistry and Faculty of health Sciences, University of Sydney, [6]Translational Neuroscience Facility, Department of Physiology, School of Medical Sciences, Faculty of Medicine, UNSW, [7]Motor Neuron Disease Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, UNSW, [8]Transgenic Animal Unit, Mark Wainright Analytical Centre, UNSW

Frontotemporal dementia (FTD) is clinically present with behavioral changes, including a loss of interest, social disinhibition and hyperactive behavior. Approximately 40% of FTD patients report a familial history of dementia, with the most frequent mutations found in the tau-encoding MAPT gene. Under physiological conditions, tau promotes and stabilizes microtubule assembly and thereby regulates their dynamics, intracellular trafficking and cell signalling. However, in disease, tau becomes abnormally phosphorylated, dissociates from microtubules and eventually aggregates into so called neurofibrillary tangles, a common neuropathological feature found in FTD patients with tau pathology.
Following the discovery of mutations in the *MAPT*- gene, transgenic mice expressing human mutant tau have been generated and used to study disease pathogenesis of the human condition. We have recently reported a detailed neuropathological and functional characterization of a novel tau transgenic mouse strain, TAU58/2, carrying the mutant human P301S mutation. The TAU58/2 mice develop early- onset motor deficits accompanied by axonal pathology prior to tau deposition, features reminiscent of human FTD with tau pathology.
In present study, we show that TAU58/2 mice develop early onset of disinhibition-like behaviour in the elevated plus maze and hyperactivity in the open field arena. Furthermore, those behavioural changes were accompanied by an early onset of tau pathology in the amygdala, characteristic features of human FTD with tau pathology.

Poster 309 – Tuesday 6th December

ALTERED COLORECTAL RESPONSIVENESS TO A COLOKINETIC AND TO PHYSIOLOGICAL STIMULI AFTER SPINAL CORD INJURY

Dr Ruslan Pustovit[1], Dr Brid Callaghan[1], Nicole F. Kerr[1], Mitchell Ringuet[1,2], Claudio Pietra[3], Dr John Furness[1,2]

[1]University Of Melbourne, [2]Florey Institute of Neuroscience and Mental Health, [3]Helsinn Preclinical Research & Development

Following a transecting spinal cord injury (SCI), there is a high incidence of constipation because the nerve pathways through which voluntary control of defecation is exerted are disrupted. It is feasible that the now neglected defecation control pathways, or the end organ, have altered sensitivity. To investigate this possibility, we recorded changes in responsiveness in rats, 5 weeks after severing the spinal cord rostral to the defecation centres, in comparison to sham operated rats. Frequencies of occurrence of propulsive contractions (>6 mmHg) were measured in anesthetized rats. At a baseline pressure of 6-10 mmHg, propulsive contractions occurred at 2.1 ± 0.8 contractions per 30 min (cont/30min) in sham rats, and 3.8 ± 0.9 cont/30min after SCI (n.s.). The centrally penetrant ghrelin receptor agonist, HM01 (0.3 mg/kg, i.v.), increased the frequency of contractions at baseline to 21 ± 4 cont/30min in sham and to 36 ± 5 cont/30min in SCI animals (both $P<0.01$ compared to before HM01). The greater effect of HM01 in SCI compared to sham was significant ($P<0.01$). Increasing pressure in the colon to 15 mmHg elicits propulsive reflexes. However, the pressure-induced increases in sham and SCI animals were not different. Behaviourally elicited defecation, by water avoidance stress, was reduced significantly, to 40% of sham, in SCI rats. These data indicate that the responsiveness of the defecation pathway to a colokinetic acting at the ghrelin receptor, but not to a distension stimulus, is heightened following SCI. Thus such colokinetics may be therapeutically effective in treating the constipation of SCI.

Poster 310 – Monday 5th December

DEPOLARIZATION-DEPENDENT SYNDAPIN I PHOSPHORYLATION IN NERVE TERMINALS

Dr Annie Quan[1], Dr Jing Xue[1], Dr Kasper Engholm-Keller[1], Dr Peter Hains[1], Professor Martin Larsen[2], Dr Mark Graham[1], Professor Phillip Robinson[1]

[1]Children's Medical Research Institute, University of Sydney, [2]Department of Biochemistry and Molecular Biology, University of Southern 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). 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. Syndapin I was dephosphorylated when nerve terminals were depolarized with 40 mM KCl, and the dephosphorylation was blocked with the calcineurin inhibitor, cyclosporin A. Therefore, syndapin I is a calcineurin substrate and a new member of the dephosphin family of proteins. Six *in vivo* phosphorylation sites were identified and sequenced using tandem mass spectrometry: Ser-76, Thr-181, Ser-343, Ser-345, Ser-346, and Ser-358. Quantitative mass spectrometry analyses on syndapin I phosphorylation indicates that only Ser-358 phosphorylation changes with KCl-dependent depolarization of the nerve terminals, while phosphorylation of the four sites, Thr-181, Ser-343, Ser-345, Ser-346, did not change. This suggests phosphorylation/dephosphorylation of syndapin could be a key signalling mechanism for controlling the function of syndapin I in ADBE, important for synaptic transmission.

Poster 311 – Tuesday 6th December

EFFECTS OF THE INDOLE DITERPENOID MYCOTOXIN LOLITREM B AND ITS PATHWAY INTERMEDIATES ON MOVEMENT AND METABOLIC STATUS IN A MOUSE MODEL OF PERENNIAL RYEGRASS TOXICOSIS.

Ms Priyanka Reddy[2], Mr Martin Combs[1], Dr Simone Rochfort[2], Dr Jane Quinn[1]

[1]School of Animal and Veterinary Sciences, Charles Sturt University, [2]Department of Economic Development, Jobs, Transport and Resources, La Trobe University

The mycotoxin lolitrem B is found in endopyte-infested perennial ryegrass (L. perenne) present in around 90% of perennial ryegrass pastures in Australia and New Zealand. Ingestion causes a neurological syndrome in grazing livestock called Perennial RyeGrass Toxicosis (PRGT) (Cunningham, 1959; Combs et al., 2014) where clinical signs include hyperexcitability, muscle tremors, ataxia ("staggers") and, in severe cases, clonic seizures and death (Combs et al., 2014). These neurological signs are thought to result from blockade of large conductance potassium channels (BK channels) by indole diterpenoid toxins, and primarily lolitrem B (Munday-Finch SC., 1997; Imlach, 2011). Despite much investigation, the relationship between systemic levels of lolitrem B and the presence or absence of its pathway intermediaries has not been well defined.
To address this question, members of the lolitrem B metabolic pathway were isolated from infested perennial ryegrass, including lolitrem B, lolitrem E and two terpendole toxins B and E, and their effects of movement quantified. Metabolomic profiling of body tissues and fluids was undertaken using HPLC-MS/MS and Quantitative Time-of-Flight analysis (LCMS/MS-QToF). Animals exposed to pure isolated lolitrem B toxin showed a significant increase in tremor and ability to maintain stability during accelerated rotarod testing, other pathway members of the family showed little or no effect. Better characterization of clinical signs and metabolic changes associated with exposure to the family of indole diterpenoid toxins present in perennial ryegrass will provide useful information for generation of grass varieties with variations in indole diterpenoid toxin production.

Poster 312 – Monday 5th December

POTASSIUM BROMIDE MITIGATES CLINICAL SIGNS OF INTOXICATION CAUSED BY THE INDOLE DITERPENOID TOXIN LOLTREM B.

Martin A. Combs[1, 2], Scott Edwards[1, 2], Allan E. Kessell[3], Adam Hamlin[4], Joshua Sherpenhuizen[1, 2], Edward Narayan[1, 2] and **Jane C. Quinn*** [1, 2]

[1]Graham Centre for Agricultural Innovation, (an alliance between Charles Sturt University and NSW Department of Primary Industries), Charles Sturt University, Pugsley Place, Wagga Wagga, NSW 2678, Australia;
[2]School of Animal and Veterinary Sciences, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW, 2678, Australia;
[3]Gribbles Pathology Adelaide, South Australia, 5065, Australia,
[4]School of Science and Technology, University of New England, Armidale, NSW 2351, Australia.
**Corresponding author: Jane Quinn jquinn@csu.edu.au*

Lolitrem B is an indole diterpenoid toxin found in perennial ryegrass. Although lolitrem B confers beneficial effects to the plant, these are counteracted by adverse effects observed animals that ingest it which can result in behavioural changes, ataxia and tremor and, in the most severe cases, seizures and death (Tor-Agbidye et al 2001). Morbidity and mortality of livestock can be in significant numbers (Cunningham 1959, Combs et al, 2014).
Currently no therapeutic treatment is available that can be delivered at a flock-wide level to mitigate the large-scale effects observed in animals during critical outbreaks. Platforms for drug testing have been difficult to establish due to seasonal variations in lolitrem B in pasture-based feeds and the difficulty in extracting this highly lipophilic toxin (Munday-Finch, 1997). To overcome this, a reproducible and reliable model of PRGT in sheep was established utilizing a feed containing high levels lolitrem B toxin. This model was compared to a rodent model where animals were exposed to pure lolitrem B toxin isolated from perennial ryegrass. Establishment of these model systems allowed a number of therapeutics to be trialled. The most significant improvements were observed in movement, behaviour and reduction of stress by administration of potassium bromide. Pharmaceutical products that could improve clinical outcomes, reduce mortality can now be tested using this experimental model. This study also shows that lolitrem B induces activation of stress pathways in the brain and identified a clear role for this toxin in for testing of pharmaceutics with anxiolytic activity.

Poster 313 – Tuesday 6th December

QUINOLINE INTOXICATION IN MILITARY MEMBERS

Dr Jane Quinn[1]

[1]School of Animal and Veterinary Sciences, Charles Sturt University

The quinoline derivatives mefloquine and tafenoquine are effective antimalarial medications (Rieckmann et al., 1974; Trenholme et al., 1975). However, despite their efficacy there is increasing evidence of adverse events resulting in both short and long-term neurological and neuropsychiatric sequelae in a subset of individuals exposed to mefloquine; the long-term effects of tafenoquine are unknown. Military organisations, including the Australian Defence Force, have utilized mefloquine since the early 1990's to protect against *Plasmodium falciparum* infection in malarious zones of military operation; tafenoquine has been trialled for terminal prophylaxis for *Plasmodium vivax* malaria as well as used to treat *P. vivax* relapse in a number of clinical cases (Nasveld and Kitchener, 2005; Elmes et al., 2008). To date, tafenoquine has only been used in TGA approved clinical trials as it is currently not registered for use in Australia. There is evidence to suggest that those who have been exposed to both mefloquine and tafenoquine may present with the most serious adverse events, including diagnosis of bipolar disorder and seizures. The long-term neuropsychiatric sequelae in these cases will be considered and presentation of clinical symptoms considered in the light of current knowledge of quinoline metabolism and their effects in the brain.

Poster 314 – Monday 5th December

LONG-TERM DEPRESSION OF EXCITATORY SYNAPTIC TRANSMISSION IN LATERAL SEPTUM

Miss Madeleine Radnan[1], Miss Chanchanok Chaichim[1], Professor Gary Housley[1], Dr. John Power[1]

[1]Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Australia

Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia
The dorsal lateral septum (dLS) is a hub in the regulation of mood and motivated behaviours. dLS neurons integrate excitatory synaptic inputs primarily from hippocampus and send inhibitory projections to a variety of brain regions involved in reward and the generation of motivated behaviour. Hippocampal-dLS synapses are plastic and high frequency stimulation has been shown to induce long-term potentiation of these synapses. Here we investigated long-term synaptic depression (LTD) at these synapses. Horizontal brain slices (400 µm) were prepared from c57/bl6 mice (5-6 wk) according to standard procedures approved by the UNSW Animal Care and Ethics Committee. Extracellular field excitatory synaptic potentials (fEPSP) were recorded using a wire electrode positioned in the caudal dLS and evoked via a bipolar stimulating electrode positioned in the fimbria fibre bundle. Low frequency synaptic stimulation (LFS; 1 Hz 15 min) depressed the fEPSP amplitude. The synaptic depression was greater in slices prepared from female mice than those from males. Immediately after LFS, the fEPSP was reduced by 50 ± 4% and 30 ± 7% in female (n = 8) and male (n = 5) mice respectively (male vs female; p = 0.009, unpaired *t*-test). After 1 h the fEPSP was reduced by 17 ± 2% in females and 2 ± 2% in males (male vs female; p = 0.0005 unpaired *t*-test). These results suggest that the cellular mechanisms underlying contextual-reward associations may be gender specific.

Poster 315 – Tuesday 6th December

MATERNAL IMMUNE ACTIVATION REDUCES SOMATOSTATIN AND SOMATOSTATIN RECEPTOR 2 mRNA EXPRESSION IN CORTEX AND STRIATUM

Tasnim Rahman[1,2,3], Tertia Purves-Tyson[1,2,3], Katerina Zavitsanou[1,2,3], Lauren Harms[4,5,6], Crystal Meehan[4,5,6], Ulrich Schall[3,5,6], Juanita Todd[4,5,6], Deborah Hodgson[4,5,6], Patricia Michie[4,5,6], Cynthia Shannon Weickert[1,2,3]

[1]Neuroscience Research Australia, [2]School of Psychiatry, University of New South Wales, [3]Schizophrenia Research Institute, [4]School of Psychology, University of Newcastle, [5]Centre for Brain and Mental Health Research, University of Newcastle, [6]Brain and Mental Health, Hunter Medical Research Institute

People with schizophrenia have decreased corticostriatal dependent learning, and cortical deficits in somatostatin and somatostatin receptor 2 (SSTR2) mRNA. Maternal infection is a risk factor for schizophrenia. This is modelled in rodents using polyriboinosinic:polyribocytidilic acid (polyI:C) in pregnant dams, and produces offspring that exhibit schizophrenia-like deficits. PolyI:C exposure during late gestation is associated with cognitive deficits in offspring. We hypothesised that late polyI:C offspring would have decreased somatostatin and SSTR2 mRNA in corticostriatal regions. We measured somatostatin and SSTR2 mRNAs in brain slices of adult male offspring (postnatal day 63-91) exposed to polyI:C or vehicle at gestational day (GD) 10 or 19. Gene expression was quantified in striatal (dorsal striatum; nucleus accumbens core; nucleus accumbens shell (NAS)) and cortical (infralimbic; cingulate; auditory) subregions by *in-situ* hybridisation. Repeated-measures ANOVA (within-subjects factors: subregions; between-subjects factors: GD, treatment) revealed polyI:C treatment decreased somatostatin mRNA in striatum ($F_{(1,24)}$=4.926, p<0.05) and cortex ($F_{(1,17)}$=4.772, p<0.05), specifically in NAS ($F_{(1,24)}$ = 7.37, p < 0.05) and cingulate ($F_{(1,17)}$=9.033, p<0.01). There were significant interactions between GD and treatment for SSTR2 mRNA in striatum ($F_{(1, 24)}$=4.508, p<0.05) and cortex ($F_{(1, 22)}$=4.533, p<0.05). PolyI:C at GD19, but not GD10, decreased SSTR2 mRNA within the striatum ($F_{(1,24)}$=5.885, p<0.05) and cortex ($F_{(1,22)}$=4.490, p<0.05) compared to saline controls. Maternal infection may contribute to deficits in somatostatin and SSTR2 expression in striatal and cortical subregions. Subcortical changes in inhibitory interneuron health may contribute to behavioural changes reported in polyI:C offspring.

Poster 316 – Monday 5th December

LEAD-INDUCED ALTERATION IN VITAMIN D METABOLISM IS INVOLVED IN LEARNING IMPAIRMENT

Dr. Abdur Rahman[1], Ameena Al-Awadhi[1], Anwar Al-Harbi[1], Dr. Khalid Khan[2]

[1]College of Life Sciences, Kuwait University, [2]Faculty of Medicine, Kuwait University

Lead (Pb) toxicity and vitamin D (VD) deficiency are both known to affect brain function. The reported negative association between blood levels of Pb and VD suggests that Pb interferes with VD metabolism. We investigated (1) the effect of Pb exposure on VD metabolism and (2) the effect of VD deficiency on spatial learning and memory in rats. Wistar rat pups were exposed to 0.2% Pb-acetate via their dams' drinking water from postnatal day (PND) 1 to 21. At PND21, the pups were sacrificed; serum 25-hydroxy VD was analyzed by LC-MS/MS and tissues were analyzed for the expression of 25-hydroxylase, 1-hydroxylase and VD receptor (VDR) by Western blot (WB) and by immunohistochemistry). Pb-exposure significantly decreased the level of serum 25-hydroxy VD (p = 0.0002). It also decreased the expression of liver 25-hydroxyalse (by 18%) and the kidney 1-hydroxylase (by 22%), whereas, the brain 1-hydroxylase was not affected, as measured by WB. However, in the thalamus of Pb-exposed pups, significantly fewer number of 1-hydroxylase immunoreactive neurons were observed. The expression of VDR in the whole brain lysate was significantly increased by Pb exposure. In a separate experiment, VD deficiency during gestation and lactation in rats significantly impaired spatial learning but not memory in the Morris Water maze test. These results suggest that Pb-induced impairment of learning may involve altered VD metabolism. The mechanism of Pb-induced alteration in VD metabolism and its physiological relevance in brain development and function warrants further research.

Poster 317 – Tuesday 6th December

FUNCTIONAL PROPERTIES OF ON-OFF NEURONS IN THE DORSAL LATERAL GENICULATE OF ANAESTHETISED MARMOSETS.

Mr Abrar Rahman[1,2], Dr Alexander Pietersen[1,2], Dr Calvin Eiber[1,2], Ms Natalie Zeater[1,2], Professor Bogdan Dreher[3], Dr Sam Solomon[3,4], Professor Paul Martin[1,2]

[1]Save Sight Institute, [2]ARC centre of excellence for integrative brain function, [3]The University of Sydney, [4]University College London

Purpose: Single neurons in the parvocellular (P) and magnocellular (M) layers of the primate dorsal lateral geniculate nucleus (dLGN) are identified as being either On– or Off-centre depending on the stimulus contrast polarity (brighter or darker than background) that evokes an increase in spike rate. A subpopulation of presumably koniocellular (K) neurons however, discharge to both light increments and light decrements (On-Off cells). Here we characterise the receptive field properties of On-Off cells recorded from the marmoset LGN. **Methods:** Single neurons were recorded extracellularly in Sufentanil-anaesthetized marmosets (*Callithrix jacchus*, n=11). Visual stimuli included pulsed (200 ms) contrast increments and decrements of variable size, as well as drifting sine-wave gratings of variable spatial or temporal frequencies, direction and cone contrast. **Results and Conclusions:** Irrespective of stimulus size, On-Off cells (n=15) discharged at both positive and negative contrast pulses. Consistent with input from On-Off retinal ganglion cells, latencies of On– and Off-responses were strongly correlated (p < 0.05). Most (11/14) On-Off cells showed high achromatic contrast sensitivity and, like 'complex' cortical cells, a low ratio of phase-locked (F1) response to maintained (F0) response, to drifting gratings. The others (3/14), were characterised by linear contrast sensitivity and a high F1/F0 response ratio. Unlike P and M cells, most (6/7) On-off cells showed strong orientation bias. On-Off

cells, whose laminar location could be determined (n = 8), were all located in the K layers. We conclude that On-Off dLGN cells have cortical-like properties and constitute a distinct functional subgroup within the koniocellular pathway.

Poster 318 – Monday 5th December

CYCLING MELATONIN SYNCHRONIZES RHYTHMIC HIPPOCAMPUS-DEPENDENT SIGNALING AND MEMORY PERFORMANCE IN MICE.

MD Irina Eckardt[1], **Dr. Oliver Rawashdeh[2]**

[1]Goethe-University Frankfurt, [2]The University of Queensland

Biological clocks regulate synaptic plasticity, and modulate the efficiency of learning and memory formation in a time-of-day dependent manner. We previously reported that numerous memory-relevant signaling components cycle across a 24h period in the hippocampus, and that cycling stimuli signaling to the hippocampus drive hippocampal rhythmicity. A candidate for a temporal messenger or time cue capable of driving hippocampal rhythmicity is the circadian clock-regulated hormone melatonin. In our study we compare the role of melatonin as a driver and synchronizer of rhythmic memory-relevant hippocampal signaling, and its role on spatial working memory (WM) performance using melatonin proficient mice.
Our data show that spatial WM performance declines during the second half of the night, thus, when melatonin levels peak, as compared to early nighttime, when melatonin levels are low. To confirm causality between endogenous melatonin and nighttime WM deficits, we assessed WM performance in melatonin receptor deficient ($MT1^{-/-}/MT2^{-/-}$) mice. The data reveal that in the absence of melatonin receptors, WM performance significantly improved at nighttime when melatonin levels peak. To exclude ontogenetic compensatory mechanisms in $MT1^{-/-}/MT2^{-/-}$-mice, we also assessed WM performance in wild-type mice after surgically silencing the sympathetic tone to the pineal gland, and thus, melatonin synthesis. The operated WT-mice show a similar improvement in WM performance during the second half of the night, thus, matching observations made in $MT1^{-/-}/MT2^{-/-}$-mice.
We conclude that nighttime melatonin functions as a time cue for the hippocampus, rhythmically modulating hippocampus-dependent processes.

Poster 319 – Tuesday 6th December

INTERMITTENT HIGH FAT HIGH SUGAR FOOD CONSUMPTION DISRUPTS SOCIAL BEHAVIOURS IN ADOLESCENT RATS

Dr Amy Reichelt[1], Miss Gabrielle Gibson[2], Dr Mukesh Raipuria[1], Miss Kirsten Abbott[2]

[1]RMIT University, [2]UNSW Australia

Adolescence is a period of increased social interaction, which is critical for developing appropriate adult behaviour. Social interaction is rewarding and is influenced by dopamine signaling in the prefrontal cortex (PFC). High fat and high sugar (HFHS) foods activate the mesocorticolimbic dopamine system and overconsumption of these foods disrupts aspects of behaviour. However, the effects of HFHS diets on social behaviours in adolescent rats is unknown. We examined the effect of 2h daily access to high fat / high sugar pellets (HFHS, 18.4kJ/g, 36% sucrose, 35% fat) beginning at postnatal day (P) 28 on social behaviours in adolescent rats. After 2 weeks of diet access social interaction (P42/43) and social memory (P45) was examined in control and HFHS consuming rats (N=8 per group). We found that HFHS consuming rats spent significantly less time interacting with the novel rat 25h post HFHS diet (P<0.05), but not 1h after HFHS diet access. In a social memory test, rats consuming HFHS diets were impaired at discriminating between a novel and familiar animal (P=0.23). No group differences were observed in levels of sociability in the social memory test or in preference of a social odour (soiled bedding). Increased levels of ΔFosB expression in the PFC of HFHS rats relative to controls (P=0.002) was indicative of chronic dysregulation of neuronal activity. Our data indicate that HFHS diets disrupt aspects of social behaviour in adolescent rats. This has translational implications for social conduct in young people who regularly consume high fat and high sugar foods.

Poster 320 – Monday 5th December

EFFECTS OF EXERCISE AND ENVIRONMENTAL ENRICHMENT IN SEROTONIN SIGNALLING MUTANT MICE

Dr Thibault Renoir[1], Mr Jake Rogers[1], Mrs Shanshan Li[1], Dr Feng Cheng[1], Prof Leonid Churilov, Prof Paul Adlard[1], Dr Laurence Lanfumey[2], Prof Maarten van den Buuse[3], Prof Anthony Hannan[1]

[1]Florey Institute of Neuroscience and Mental Health, University of Melbourne , [2]INSERM UMR S894, [3]La Trobe University

Clinical evidence indicates that serotonin-1A receptor ($5\text{-}HT_{1A}R$) and serotonin transporter (5-HTT) gene polymorphisms are associated with anxiety/depressive disorders and cognitive impairment. In animal models, exercise and environmental enrichment (EE) can change emotionality-related behaviours as well as enhance some aspects of cognition and hippocampal neurogenesis. We investigated the effects of exercise and EE on cognition as well as anxiety- and depression-like behaviours in wild-type and mutant mice.
Using an algorithm-based classification of search strategies in the Morris water maze, we reported that exercise increased the odds for mice to select more hippocampal-dependent strategies. In the retention probe test, exercise (but not EE) corrected long-term spatial memory deficits displayed by $5\text{-}HT_{1A}R$ knock-out (KO) mice. In agreement with these findings, only exercise increased hippocampal cell survival and BDNF protein levels. Similarly, the depressive-like behaviours displayed by 5-HTT KO mice was rescued by exercise only.

Interestingly, those exercise-induced antidepressant-like effects seemed to be independent of adult hippocampal neurogenesis, but correlated with rescued long-term potentiation in the hippocampus of exercised 5-HTT KO mice.
Finally, only EE (but not exercise) reduced anxiety-like behaviours, demonstrating dissociation between improvements in cognition and innate anxiety.
Together, these results demonstrate differential effects of exercise versus EE in mouse models of anxiety/depression with cognitive impairment. Overall, 5-HT_{1A}R and 5-HTT do not seem to be necessary for those behavioural effects to occur. These findings will have implications for our understanding of how exercise and environmental enrichment enhance experience-dependent plasticity, as well as their differential impacts on anxiety and cognition.

Poster 321 – Tuesday 6th December

THE NEURONAL AND PERCEPTUAL EFFECTS OF VISUAL MASKING

Ms Katrina L Richards[1], Dr Dasuni S Alwis[1,2], Professor Ehsan Arabzadeh[3], Dr Nicholas SC Price[1]

[1]Monash University, [2]Florey Institute of Neuroscience and Mental Health, [3]Australian National University

Visual masking describes the reduction in the perception of a target stimulus by a preceding (forward masking) or succeeding (backward masking) stimulus. In this way, masking illustrates a disconnect between the physical stimulus, its neuronal representation, and its percept. To understand the neural correlates of masking, we examined how visual masks affect orientation discrimination in awake rodents, and separately examined neuronal responses to similar stimuli in the primary visual cortex (V1). Long-Evans rats (n=6) were trained to discriminate the orientation of a Gabor patch presented for 42 ms. These target stimuli were presented at stimulus onset asynchronies (SOA) of –250 to 250 ms relative to an uninformative mask. In a separate cohort of halothane-anaesthetised animals (n=28) neuronal responses to target and mask stimuli were recorded from all layers of V1 using a 32-channel linear array. Early and late components of the neuronal response were analyzed separately. Behaviourally, for both forward and backward masking conditions, discrimination performance was impaired when the target and mask occurred close in time (i.e. short SOAs). Similarly, across all cortical layers, neuronal firing rate and orientation selectivity decreased monotonically with shorter SOAs. However, within early and late response components the reductions in orientation selectivity were not reliably predicted by changes in firing rate. Collectively, our data suggests that changes in V1 activity are responsible for the impairment in perceptual discrimination during masking. We propose that neural integration and lateral inhibition may explain the perceptual effects of monotonic visual masking.

Poster 322 – Monday 5th December

DECODING THE VALUE OF JUICE FROM ELECTROCORTICOGRAPHIC SIGNALS IN MONKEY PREFRONTAL CORTICES AND ITS MODULATION THROUGH THE DECODED NEUROFEEDBACK.

Shingo Tanaka[1], Keisuke Kawasaki[2], Isao Hasegawa[2], Takafumi Suzuki[3], **Masamichi Sakagami[1]**

1. Brain Science Institute, Tamagawa University, Tokyo, Japan. 2. Department of Physiology, Niigata University School of Medicine, Niigata, Japan. 3. Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, Japan

To examine dynamic signal processing related to value-based decision-making, we recorded local field potentials (LFPs) from electrocorticographic (ECoG) electrodes implanted in the left lateral prefrontal cortex (LPFC), the left orbitofrontal cortex (OFC) and both side of the medial prefrontal cortex (MPFC), while two monkeys performed the free-choice task with 6 different juices. Using the wavelet power and phase timing in six frequency domains (δ, θ, α, β, low-γ, high-γ) we decoded the values of the juices with Sparse Linear Regression (SLiR) algorithm. Decoded values from 3 areas were significantly correlated with the behaviorally estimated values. Also, to examine the causal role of prefrontal value related signals, we applied the decoded neurofeedback (DecNef) technique to modulate the value of juice. We could successfully increase the decoded value of less preferred juice and decrease that of more preferred juice. Accordingly values of juices estimated from behavior were changed in consistent directions.

Poster 323 – Tuesday 6th December

DEVELOPMENT OF A MODEL OF CHRONIC INFLAMMATION IN THE MURINE VAGINA

Ms Harman Sharma[1], Ms Esther Ji[1], Mrs Pat Vilimas[1], Miss Melinda Kyloh[2], Mr Nick Spencer[2], Ms Christine Barry[1], Mr Rainer Viktor Haberberger[1]

[1]Department of Anatomy & Histology and Centre for Neuroscience, School of Medicine, Flinders University of South Australia , [2]Department of Human Physiology and Centre for Neuroscience, School of Medicine, Flinders University of South Australia

Impairment of nerve fibres in vaginal tissue following inflammation is implicated in conditions such as vulvodynia and vestibulodynia and it is important to define the underlying pathophysiology and subsequently find an effective treatment. To achieve this, it is essential to develop a robust and reliable disease model. There is still an unmet need for a reproducible and efficient mouse model of vaginal inflammation. We aimed to establish a reproducible model of chronic vaginal inflammation by injecting the pro-inflammatory agent, Complete Freund's Adjuvant (CFA) (5 □L) into the vaginal wall of mice (n=4). After 7 days, mice were euthanized the vaginal tissues were dissected, evidence of inflammation visible under the dissecting microscope was assessed and the tissue processed for

immunohistochemistry. We established parameters for evaluating a controlled chronic mild inflammation. In all animals, injection with CFA caused mild inflammation in all animals indicated by morphological signs such as oedema, redness and swelling. In addition, histochemistry showed substantially increased vascularisation in the adventitia and lamina propria layers of the vaginal wall. Immunohistochemistry, demonstrated significantly increased CD-68 labelling in the vulvar region of the chronic inflamed vagina compared to naïve mice (n=3 per group; t-test, $p < 0.005$). These results suggest that this novel model of inflammation in the mouse vagina is robust and reproducible. Further studies will involve investigating dorsal horn neuron activity in response to stimulation in the CFA-induced vaginal inflammation model.

Poster 324 – Monday 5th December

ALTERED CHOLESTEROL HOMEOSTASIS IN SPINAL CORD OF OLD MICE

Dr Gemma Parkinson[1,2,3], Mark Bigland[1,2,3], Mitchell Cummins[1,2,3], Dr Dedreia Tull[4], **Dr Doug Smith[1,2,3]**

[1]Neurobiology of Ageing and Dementia Laboratory, School of Biomedical Sciences and Pharmacy, University Of Newcastle, [2]Priority Research Centre for Brain and Mental Health Research, University of Newcastle, [3]Hunter Medical Research Institute, [4]Metabolomics Australia, University of Melbourne

Nervous system ageing can lead to profound functional changes that can impact an individual's quality of life. To better preserve function we need to understand how the ageing process affects the nervous system. We are carrying out genomics investigations of various CNS regions of young (<3 mos) and old (>24 mos) mice. Our RNA-Seq analysis of spinal cord indicated a global down-regulation of cholesterol synthesis, with expression of 19 of the 22 synthesis-related genes significantly lower in old animals. A subset of these changes was confirmed by qPCR. We then investigated age-related changes in other cholesterol – related genes and found decreased expression for the cellular uptake receptor (*Ldlr*), but increased expression for extracellular transport (*ApoE*), intracellular transport (*Npc2*), cell export (*Abca1*), esterification (*Acat1*), and hydroxylation (*Ch25h*). These age-related gene expression changes indicate cells are attempting to restore cholesterol balance. To gain further insight into how ageing affects cholesterol homeostasis, we carried out a lipidomics analysis (GC/LCMS) of spinal cords. Paradoxically, free cholesterol levels were significantly increased in spinal cords from old mice, although the synthesis intermediate, lathosterol, was markedly down. There were also significant increases (range: 2 - 112 fold) in all 25 cholesterol esters detected. These genomic and lipidomic data are consistent with the notion that the aged spinal cord is trying to deal with excess free cholesterol – a potentially toxic lipid.

Poster 325 – Tuesday 6th December

CHANNEL RHODOPSIN ASSISTED CIRCUIT MAPPING OF CALRETININ POSITIVE NEURONS IN DORSAL HORN MICROCIRCUITS

Kelly M Smith[1], Dr David I Hughes[2], Dr Phillip Jobling[1], Proff Robert J Callister[1], A/Proff Chris V Dayas[1], A/Proff Brett A Graham[1]

[1]University of Newcastle, [2]University of Glasgow

The spinal dorsal horn is a key region in the processing pathway for sensory information. Substantial neuron diversity exists in this region, making identification and subsequent analysis of discrete neuron populations difficult. In this study we have used channel rhodopsin assisted circuit mapping to identify specific connections of a population of dorsal horn interneurons expressing the calcium binding protein calretinin (CR). Local circuit connectivity was assessed in the slice preparation using 1ms pulses of blue light (490nm) delivered at 0.1Hz. Excitatory synaptic connections (CNQX sensitive) were observed in 27/33 recordings from CR positive neurons and 16/18 CR negative neurons. Excitatory synaptic connections were also observed in all recordings from CR negative neurons in lamina 1 (9/9). Additionally, we have developed a method to assess the role of CR positive dorsal horn neurons in an intact, behaving preparation. Briefly, CRChR2 animals were anaesthetised and paraspinal musculature retracted around the L4 spinal cord segment. A fibre optic probe is secured between the T12 and T13 vertebra and the animal is allowed to recover for 1 week. Consistent with our in vitro data showing CR connections with lamina I, activating CRChR2 neurons in this preparation produces strong nocifensive behavioural response in all animals (n=12). Together these data suggest an important role for CR positive interneurons in the transmission of noxious sensory signals. Their strong connectivity within the dorsal horn places them in an ideal position to alter incoming sensory information prior to its relay to higher brain centres by lamina I projections neurons.

Poster 327 – Tuesday 6th December

WIRELESS OPTOGENETICS: SIMULTANEOUS ACTIVATION OF MULTIPLE LIGHT EMITTING DIODES (GREEN AND BLUE) FOR ACTIVATION OR INHIBITION OF MULTIPLE DIFFERENT NEURAL PATHWAYS

Kelley N[1] & Spencer NJ[2]

[1]Biomedical Engineering, Flinders Medical Center, South Australia & [2]Department of Human Physiology, School of Medicine, Flinders University of South Australia, South Australia

A major recent advance in optogenetics is the ability to provide untethered (wireless) activation of miniature light emitting diodes (LEDs) to activate opsins. Our aim was to determine whether multiple LEDs can be simultaneously illuminated wirelessly, using both green and blue LEDs and if so, is there any reduction in light output. We used a 1.5 GHz resonant cavity to power miniature LEDs. Light power output

was measured by affixing a 1mm diameter optical fibre to form a measurement probe. Extra receivers(with LEDs) were placed in line along the axis of their receiving coils at 4.5mm spacing, first one to the left, then one to the right, then a second one on the right to give four receivers in a row. Light power output was recorded for each configuration. Mean values of the percentage drop in original light output were plotted, as extra receivers (with LEDs) were added. With 2 LEDs activated, there was no significant drop in light intensity (2.4% decrease; P=0.16;N=3) from a mean of 41.9μW to a mean of 40.9 μW. With 4 LEDs activated there was a significant drop in light output by 10.3%; P=0.003; N=3). Green and blue LEDs could be activated simultaneously. We demonstrate the wireless optogenetics can be used to simultaneously activate multiple LEDs (green and blue). This means that in conscious untethered animals, we can inhibit and/or excite multiple neural pathways simultaneously at different sites in the animal. This is the first demonstration that green LEDs can be activated wirelessly.

Poster 328 – Monday 5th December

REPEATED STRESS EXPOSURE INDUCES HIPPOCAMPAL NITROSATIVE STRESS AND UPREGULATES PROTECTIVE ANTIOXIDANT GENES IN THE RAT

Dr Jereme Spiers[1], Hsiao-Jou Cortina Chen[1], Mr Johnny Lee[1], Mr Tsz Yip[1], A/Prof Conrad Sernia[1], Dr Nickolas Lavidis[1]

[1]The University Of Queensland

Hippocampal neuronal nitric oxide synthase (NOS) derived-nitric oxide, a free radical with both physiological and pathological functions, has recently been shown to mediate chronic stress-induced depressive-like behaviour in rodents. However, we have previously demonstrated that acute stress decreases neuronal NOS expression in the hippocampus despite increased concentrations of nitric oxide metabolites and fluorescent indicators of nitrosative status which may suggest feedback inhibition of neuronal NOS expression. Therefore, in the present study we have utilised markers of oxidative/nitrosative stress and members of the antioxidant nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway to observe the changes occurring in the hippocampus following repeated restraint stress exposure. Male Wistar rats were subject to control conditions or 6 hours of restraint stress applied for 1, 2, or 3 days (n=8 per group) after which the hippocampus was isolated for nitrosative assays and relative gene expression. The hippocampus showed increased nitrosative status and nitric oxide metabolites, indicative of higher nitric oxide. However, neuronal NOS decreased over all stress treatment groups, while increases were observed in inducible NOS and xanthine dehydrogenase, a NOS independent nitric oxide generator. In addition to inducible NOS, expression of other inflammatory markers including interleukin-6 and interleukin-1β also increased despite high concentrations of anti-inflammatory glucocorticoids. Moreover, there were transient increases in expression of antioxidant members of the Nrf2 pathway. Together, these results demonstrate that inducible NOS and alternative NOS-independent sources contribute to nitric oxide production which may subsequently decrease neuronal NOS expression to induce an inflammatory and antioxidant response in the hippocampus.

Poster 329 – Tuesday 6th December

STIMULATING GLIAL CELLS – A NOVEL NEURAL REPAIR STRATEGY

Dr Marie Vial[1], Dr Johana Tello Velasquez[1], Mr Mo Chen[1], A/Prof Rohan Davis[1], A/Prof Jenny Ekberg[3], **Dr James St John[1,2]**

[1]Eskitis Institute for Drug Discovery, Griffith University, [2]Menzies Health Institute Queensland, Griffith University, [3]Faculty of Health Sciences and Medicine, Bond University

In Australia, over 12,000 people live with a spinal cord injury and around 400 new cases are reported every year. Glial cells have been used as promising candidates for cell-based therapies in neural repair. However, low cell proliferation and slow cell migration reduce the integration of transplanted cells into the injury site leading to diminished neural regeneration. The use of neurogenic and neurotrophic factors has long been considered as a potential therapeutic approach, however due to their high molecular weight, most endogenous factors are unstable and unable to cross the blood-brain barrier. We have commenced the screening of natural compound libraries to identify compounds that stimulate the activity of glial cells. We have found natural compounds that stimulate specific activities of cells, and which are specific to different types of glial cells. For the peripheral glia olfactory ensheathing cells and Schwann cells, the compound curcumin stimulated phagocytic activity of OECs by 20-fold over 12 hours ($p<0.001$), but had no significant effect on the phagocytic activity of Schwann cells. The natural product RAD288 (3 μM) stimulated the proliferation of OECs by 120% ($p<0.001$) increased migration by 205% ($p<0.001$) over 24 hours, whereas the structurally similar compound RAD289 stimulated proliferation of OECs by 150%, but do not stimulate migration. These results demonstrate that specific activities of glial cells can be potently stimulated by small molecule compounds and that the effects can be cell-type specific. Thus the screening of natural compounds can be a rich source of discovery of compounds with therapeutic potential.

Poster 330 – Monday 5th December

THE MERTK PHAGOCYTIC RECEPTOR IN ASTROCYTES REGULATES SYNAPSE DENSITY AND BEHAVIOUR IN THE ADOLESCENT CNS

Dr. Jerome Staal[1], Mr. Eddy Yang[1], Dr. Thibault Renoir[1], Prof. Trevor Kilpatrick[1,2]

[1]Florey Institute Of Neuroscience And Mental Health, [2]2. Melbourne Neuroscience Institute

Astrocytes are the most abundant glial cell type in the central nervous system (CNS) and are intricately associated with synaptic function. Despite growing gene expression studies highlighting the presence of phagocytic receptors in astrocytes from the healthy brain, little is known about the phagocytic role of these cells outside of injury and disease. A recent study demonstrated the key role phagocytic receptors play in the early developmental pruning of synapses in visual associated brain areas and suggest that astrocytes may contribute to this refinement. Yet there is little known regarding the specific role of astrocyte phagocytic activity in CNS synaptic pruning, which may be distinct to microglia.
To study this, we conditionally deleted the phagocytic receptor MerTK in astrocytes through the expression of Cre-recombinase under the glial fibrillary acidic protein (GFAP-cre^{ERT2}). Importantly, the Cre-dependent deletion of MerTK only occurs following administration of tamoxifen, which was delivered to 5-week-old adolescent animals by oral gavage. Brains were harvested for histological and western blotting analysis 14 days after tamoxifen.
Deletion of the phagocytic MerTK receptor in astrocytes resulted in excess pre-synaptic proteins ($P<0.05$) in the hippocampus of adolescent animals compared to vehicle controls and altered behavioral function. Specifically, MerTK deletion resulted in significantly reduced social interactions (three-chamber test), reduced marble burying and altered pre-pulse inhibition.
In conclusion, we demonstrate that specific deletion of the phagocytic MerTK receptor in astrocytes of adolescent animals results in significant alterations in synaptic refinement and the subsequent development of behavioral features associated with autism and schizophrenia.

Poster 331 – Tuesday 6th December

MALDI IMAGING MASS SPECTROMETRY PROFILING OF PROTEIN CHANGES IN SPINAL CORDS FROM MURINE MODEL OF NEUROPATHIC PAIN.

Mrs VASILIKI STAIKOPOULOS[1], Dr Arun Everest-Dass[2], Ms Parul Mittal[3], Mr Matthew Briggs[3], Professor Peter Hoffmann[3], Professor Nicolle Packer[2], Professor Mark Hutchinson[1]

[1]ARC Centre of Excellence for Nanoscale Biophotonics, University Of Adelaide, [2]ARC Centre of Excellence for Nanoscale Biophotonics, Macquarie University, [3]Adelaide Proteomics Centre, University of Adelaide

Understanding the molecular mechanisms underlining the development and maintenance of neuropathic pain is important to the development of therapies to help manage this condition effectively. Histological analysis of protein abundance and distribution has contributed to this understanding but is often limited by the selective antibody targeted approach and doesn't allow for a global analysis of several protein changes. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) of *in-situ* trypsin cleaved proteins combines the sensitivity and selectivity of mass spectrometry with 2D spatial analysis to provide a new dimension over conventional histological analysis to provide unbiased visualization of the distribution of proteins.
In this study, MALDI-IMS was used to identify differentially expressed proteins from formalin-fixed paraffin embedded (FFPE) mouse spinal cord tissue from chronic constriction injury and sham animals. Several peptide *m/z* (for example 968.4, 980.4, 1045.5, 1067.4, 1085.1) species were identified to be significantly different spatially and semi-quantitatively between allodynic versus control mice (ROC analysis, AUC > 0.7). Critically these differences were observed in the dorsal horn of the spinal cord, a key somatosensory nuclei of the spinal cord.
These data are the first example of the use of MALDI-IMS in the exploration of the aetiology of chronic pain. The identification of the peptide sequence and its protein ID are orthogonally characterised using liquid-chromatography mass spectrometry analysis of concomitant FFPE tissue sections. Additional validation of the peptide target and its mechanistic role in the presentation of exaggerated pain behaviours is continuing.

Poster 332 – Monday 5th December

GHRELIN RECEPTORS IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS ARE REQUIRED TO DETECT AND COUNTER HYPOGLYCEMIA

Dr Romana Stark[1], Vanessa Bartolomeo[1], Natalie Guiney[1], Assoc. Prof. Dr Zane Andrews[1]

[1]Monash University

The ventromedial hypothalamus (VMH) has emerged as a critical brain region that senses hypoglycemia and engages counter-regulatory mechanisms to restore normal glycaemia. Indeed, sensing and responding to hypoglycaemia is the number 1 concern for patients with type 1-diabetes. Moreover, there is a greater need to regulate blood glucose during metabolic states of energy deficit and the gut hormone ghrelin informs the brain of energy deficit by binding to ghrelin receptors (GHSR) in the brain. Therefore, we questioned whether GHSRs on VMH neurons prevent hypoglycaemia by initiating appropriate counter-regulatory responses.
Temporal ablation of GHSRs in the VMH was achieved by injecting AAV-flex-taCaspase-TEVp into 8-10 week old GHSR-ires-cre mice. This method expresses a modified caspase variant that killed GHSR-expressing VMH neurons after 4 weeks. Caspase-induced cell death of GHSR-VMH neurons reduced refeeding after fasting, lowered fasting blood glucose, reduced blood glucose production in response to 2-Deoxyglucose (2DG)-induced hypoglycemia and increased glucose clearance during an oral GTT. Behavioral tests show increased anxiety-like behaviour and reduced motivation for food rewards.
Next, we injected excitatory hM3Dq 'designer receptors exclusively activated by designer drugs' into GHSR-ires-cre mice to remotely stimulate GHSR-expressing neurons in the VMH. The activation of GHSR-VMH neurons increased 5-hour food intake, increased plasma glucose in response to 2DG-induced hypoglycemia and restored blood glucose faster than saline control in response to insulin-induced hypoglycemia.

These studies demonstrate that GHSR-expressing neurons in the VMH are critical to sense hypoglycemia and mount an appropriate counter-regulatory response in an attempt to restore normal glycemia.

Poster 333 – Tuesday 6th December

MID-LIFE NOVEL ENVIRONMENTAL ENRICHMENT EXACERBATES AB NEUROPATHOLOGY IN APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE

Miss Kimberley Stuart[1], Dr Anna King[1], Dr Mathew Summers[2], Professor James Vickers[1]

[1]Wicking Dementia Research and Education Centre, University of Tasmania, [2]School of Social Sciences, University of the Sunshine Coast

Objective The current project investigates the potential for later-life environmental enrichment (EE) to reduce Aβ burden and increase synaptic contacts in ageing and in a mouse model of early-stage AD.
Methods Male transgenic (APPswe, PSEN1dE9, *n* = 38) and Wt mice (*n* = 37) entered differential housing from 6 – 12 months of age. Mice entered standard (SH) or EE housing. EE comprised a cage double the size of the SH cage, with various enrichment objects. A sub-set of EE mice (EE+) received complex/novel stimulation by entering a novel cage three times weekly.
Results APP/PS1 mice housed in EE+ showed increased hippocampal fibrillar Aβ plaque burden (M = 2.70%) compared to SH (M = 1.51%) or EE (M = 1.98%) mice (*p* = .02). In addition, they had significantly increased blood corticosterone levels (SH M = 124.59; EE M = 133.03; EE+ M = 216.29 ng/mL, *p* = .01). APP/PS1 EE+ mice demonstrated no significant cognitive differences to those in SH or EE despite increases in Aβ pathology and corticosterone. In addition, Wt EE+ mice did not show this pattern of heightened corticosterone.
Conclusions Later-life EE+ in APP/PS1 mice was associated with increased corticosterone levels and exacerbated Aβ neuropathology. The findings indicate that this kind of chronic stressor may be a significant contributing factor in the progression of AD. Synaptic alterations in cortical and hippocampal regions will be analysed in order to determine potential compensatory mechanisms to account for intact cognitive function despite increased pathological burden in EE+ APP/PS1 mice.

Poster 334 – Monday 5th December

THE CONTROL OF FIRING PATTERN OF MIDBRAIN PERIAQUEDUCTAL GRAY NEURONS IN VIVO.

Dr Hari Subramanian[1]

[1]Queensland Brain Institute

The midbrain periaqueductal gray (PAG) is a relay of the limbic brain and generates motor programs to mediate environmental challenges (flight/freezing) or emotional expression (vocalization, fear, stress/anxiety). In this context, the PAG maintains critical circuitries that control respiration, vocalization, cardiovascular system, pain, micturition and lordosis1. However, activity patterns of PAG neurons *in vivo* are not known. In urethane-anesthetized rats PAG was stereotaxically mapped for neuronal function. The anterior cingulate cortex (ACC) involved in a range of emotional processing was chemically stimulated to examine its effect on PAG neuronal function. PAG remained predominantly quiescent in the resting state. Non-firing PAG cells were activated by iontophoresis of the excitatory amino acid glutamate and ceased activity when glutamate ejection was terminated. Sporadically firing cells were found in dorsal PAG. Robust spontaneously active cells were very few, restricted to the lateral and ventrolateral PAG. These were either non-bursting cells with a 200 to 250 msec near-normal distribution or burst-firing cells showing bimodal distribution. Stimulation of ACC caused activation of PAG neurons. In such instances, PAG cells showed two types of of activity patterns; single spike firing and burst firing, phasic or tonic. The functional implications of PAG neuronal activity are discussed in terms of inputs from ACC and its impact on overall descending motor and autonomic control.

1. Subramanian HH and Holstege G (2014). The midbrain periaqueductal gray changes the eupneic respiratory rhythm into a breathing pattern necessary for survival of the individual and of the species. *Prog. Brain Res*. 212:352-384.

Poster 335 – Tuesday 6th December

BRAIN EXTRACTS DERIVED FROM PART AND AD INDUCES TAU PATHOLOGY IN THE HIPPOCAMPUS OF P301S MUTANT TAU TRANSGENIC MICE.

Lisa Suh[1,2], Dr. Arne Ittner[1], Dr. Greg Sutherland[2], Prof. Jillian Kril[2], Prof. Lars Ittner[1,3]

[1]Dementia Research Unit, University of New South Wales, Kensington, NSW 2052, Australia, [2]Discipline of Pathology, Sydney Medical School, The University of Sydney, Camperdown, NSW 2006, Australia, [3]Neuroscience Research Australia, Randwick, NSW 2031, Australia

The accumulation of amyloid-beta as plaques and tau as neurofibrillary tangles are among the classic pathological features of Alzheimer's disease (AD) with the tau burden being most congruent with disease progression. In contrast, Primary Age-Related Tauopathy (PART) represents a subset of individuals with AD-like tau pathology who had normal or only mild cognitive impairment at death. With growing evidence that pathological tau in AD is capable of propagating across neuroanatomical connections, our objective was to investigate the potential of PART versus AD brain tissue in accelerating tau pathology in P301S mutant tau transgenic TAU58/2+ mice. Briefly, brain extracts

derived from the entorhinal cortex of AD, PART or control donors were injected unilaterally into the hippocampus and cortex of two months old recipient transgenic TAU58/2+ mice. All mice were monitored for three months prior to histological analysis. For all treatment groups, increased tau pathology was seen in the injected hippocampus compared to the contralateral side. Further, there was a step-wise increase in pathology, with the AD treatment group showing the highest tau burden, the PART treatment group showing a moderate tau burden, and the control group showing a low tau burden not significantly different to sham-injected animals. Together, these findings suggest that pathological tau, or a combination of tau and other factors such as amyloid beta in the AD brain, is a more potent inducer of tau pathology than tau found in PART in susceptible mice.

Poster 336 – Monday 5th December

THE TEMPORAL EFFECTS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) ON MOTOR PERFORMANCE

Mr Eurwin Suryana[1], Mrs Amelia Sedjahtera[2], Mrs Lydia Gunawan[2], Mrs Kali Perronnes[2], Professor David Finkelstein[2], Associate Professor Kay Double[1]

[1]. Discipline of Biomedical Science, Sydney Medical School, Brain and Mind Centre, The University of Sydney, [2]The Florey Institute of Neuroscience and Mental Health, The University of Melbourne

MPTP intoxication of mice is a commonly used model of Parkinson's disease, however the reported effects on motor performance are varied. The aim of the study was to elucidate the temporal effects of MPTP on motor performance of wild-type (WT; C57bl6), and copper-deficient (Ctr1 knock-down) mice. We treated cohorts of WT mice with MPTP (60mg/kg) or saline, and investigated pole test performance 5, 12, and 19 days post-injection, as well as DigiGait posture and gait measurements at 6, 13 and 20 days. Copper-deficient mice were also treated with MPTP (60mg/kg) or saline, and undertook pole test 19 days post-injection, and DigiGait measurements 20 days post-injection. Five days post-injection, WT mice took longer to complete the turn component of the pole test (p=0.009), with total test time also being longer in these animals (p=0.009). By days 12 and 19, pole test performance of MPTP-treated mice was equivalent to saline-treated control mice. DigiGait analysis did not reveal any significant differences in gait dynamics or posture of MPTP-treated mice. Further, motor performance in both the pole test and DigiGait was not altered in the copper-deficient MPTP- and saline-treated control mice. MPTP induces specific motor deficits that are revealed by pole test, but not DigiGait analysis, and these deficits are apparent 5 days post-lesion but recover over time. This study identifies the specific nature of motor performance deficits, and the temporal pattern of MPTP treatment, highlighting the importance of choosing appropriate behavioural testing days when designing MPTP-based experiments utilising motor performance as an endpoint.

Poster 337 – Tuesday 6th December

A COMPARISON OF THE HISTOLOGY AND NEUROINFLAMMATORY RESPONSE AFTER SPINAL CORD INJURY IN ADULT AND INFANT RATS SHOWS SIGNIFICANT DIFFERENCES

Ms Theresa Sutherland[1], Ms Alison Ricafrente[1], Dr Catherine Gorrie[1]

[1]University Of Technology Sydney

Spinal cord injury (SCI) is a devastating condition resulting in loss of tissue, functional impairment and exhibits only limited repair. To date research has focused on abetting the degenerative secondary injury phase and promoting tissue repair. The immune response to SCI can be both beneficial and detrimental to recovery, playing an important role in the progression of the secondary injury. There exists a trend for a better functional recovery in younger patients, compared to adults, which is also reported for animal studies, however the reasons for this are yet to be elucidated.
Using a mild contusion injury model adult (9wk), and infant (P7) Spague-Dawley rats were compared histologically at 24hrs, 1wk, 2wks and 6wks post-injury (n=108) to examine the injury progression. The neutrophils and macrophages/microglia were also quantitated. Further animals (n=60) were assessed using flow cytometry to quantitate phenotypes of macrophages, neutrophils and T-Cells; as well as for multiplex cytokine ELISA.
The results showed significantly different injury pattern, and a decreased inflammatory response, in the infants at both acute and chronic time points. The flow cytometry and multiplex ELISA results track with the initial histochemistry and further highlight the cellular and molecular differences in the inflammatory response in young animals. This suggests that the inflammatory response is significantly different in developing and mature spinal cords. It is possible that these differences may contribute to the observed better recovery. This raises the possibility of manipulating the adult responses to resemble the infants to promote greater recovery and quality of life.

Poster 338 – Monday 5th December

DISTRIBUTION OF KEY SYNAPTIC PROTEINS IN THE ENTERIC NERVOUS SYSTEM OF MOUSE COLON

Miss Mathusi Swaminathan[1], Dr. Jaime Foong[1], Dr. Elisa Hill-Yardin[1], Prof. Joel Bornstein[1]

[1]The University of Melbourne

Large arrays of synaptic proteins regulate the function of synapses. Synaptic protein mutations or defects alter synaptic function and underlie several neurodevelopmental and neurodegenerative disorders. The enteric nervous system (ENS) and central nervous system

express similar synaptic proteins, but these are poorly characterised in the ENS. We aimed to identify the distributions of synaptic proteins in the ENS of the mouse colon using immunohistochemistry. We examined synaptic contacts (varicosities) onto specific enteric neuronal subclasses using high-resolution confocal micrographs reconstructed with Imaris software (Bitplane). The distribution of presynaptic proteins (synaptophysin and synaptotagmin-1) within the ENS was examined by quantifying colocalization (represented as Mander's coefficients) with markers for excitatory (vesicular acetylcholine transporter, VAChT) and inhibitory (neuronal nitric oxide synthase, nNOS) nerve terminals using Volocity software (Perkin Elmer). We found that a single nNOS+ neuron can receive up to 160 synaptophysin+ varicosities. Synaptophysin and synaptotagmin-1 co-localized with most VAChT+ varicosities (Mander's coefficients of 0.50±0.03 and 0.64±0.03 respectively (3 animals, 0 no overlap; 1 100% overlap)). There was minimal colocalization with, nNOS+ (Mander's coefficients of 0.29±0.02 for synaptophysin and 0.27±0.03 for synaptotagmin-1 (3 animals)). This suggests that cotransmitter release from nNOS+ varicosities may not involve synaptophysin and synaptotagmin-1. We also found expression of the post-synaptic protein PSD-93 in the cytoplasm, dendrites and axons of most enteric neurons, including 61±6% of nNOS+ (3 animals) and 68±8% of choline acetyltransferase+ (2 animals) neurons. Overall, this study suggests that pre and postsynaptic proteins found in the brain are selectively expressed in mouse ENS.

Poster 339 – Tuesday 6th December

EFFECTS OF ANAESTHESIA ON MOTOR EVOKED POTENTIALS INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN RATS: IMPLICATIONS FOR PLASTICITY STUDIES

Mr M Sykes[1,2,3], Miss Natalie Matheson[1,2], Dr Philip W Brownjohn[1,4], Mr Alexander Tang[3], Dr Jennifer Rodger[3], Dr Jonathan Shemmell[1,4], Associate Professor John Reynolds[1,2]

[1]Brain Health Research Centre and Brain Research NZ Centre of Research Excellence, [2]Department of Anatomy, University of Otago, [3]Experimental and Regenerative Neuroscience, School of Animal Biology, University of Western Australia, [4]School of Physical Education, Sport and Exercise Sciences, University of Otago

Repetitive transcranial magnetic stimulation (rTMS) is primarily used in humans to change the state of cortical excitability, using Motor Evoked Potentials (MEPs) to compare stimulation protocols. Stimulation of the motor cortex produces a twitch response in a targeted muscle, the amplitude of which provides an indirect measure of the excitability of the cortex. rTMS to the motor cortex can alter MEP amplitude, however results are highly variable between studies and the mechanisms underlying any change and its locus are poorly understood, prompting the use of *in vivo* animal models. These models necessitate the use of general anaesthesia, which can affect plasticity-like mechanisms and potentially alter the effectiveness of an rTMS protocol. In the present study, we explored the effect of anaesthetic on MEP amplitude, recorded before and after the facilitatory rTMS protocol, intermittent theta burst stimulation (iTBS). MEPs were recorded in the brachioradialis muscle of the forelimb under two anaesthetics: a xylazine/zoletil combination and urethane. We found MEPs could be induced under both anaesthetics, with no differences in the resting motor threshold or the average baseline amplitudes. However, MEPs were highly variable between animals under both anaesthetics, with the xylazine/zoletil combination showing higher variability and most prominently a significant rise in amplitude across the baseline recording period. Application of iTBS did not facilitate MEP amplitude as was expected, under either anaesthetic condition. With the continued use of MEPs in humans as a means of assessing protocol efficacy, there is a continued need for animal studies to address the underlying mechanisms.

Poster 340 – Monday 5th December

THE ROLE OF THE NEURAL CELL ADHESION MOLECULE 2 (NCAM2) IN REGULATION OF SYNAPSE FORMATION, MAINTENANCE OF SYNAPSE INTEGRITY AND REGULATION OF NEURONAL EXCITABILITY

Dr Vladimir Sytnyk[1], Dr. Iryna Leshchyns'ka[1], Lifu Sheng[1]

[1]University Of New South Wales

The neural cell adhesion molecule 2 (NCAM2) is a large glycoprotein expressed at the cell surface of neurons, which is encoded by a gene on chromosome 21 in humans. NCAM2 belongs to the immunoglobulin superfamily of cell adhesion molecules due to the presence of five immunoglobulin domains, which play a key role in formation of adhesion bonds between cells. NCAM2 is over-expressed in brains of Down syndrome patients. Ablation of NCAM2 expression alone also results in intellectual disability in humans. The functions of NCAM2 in the brain remain, however, poorly understood.
We have shown that NCAM2 accumulates in synapses between neurons in the mouse and human brain. Disruption of NCAM2-dependent synaptic adhesion results in synapse disassembly in the hippocampus affected by Alzheimer's disease, indicating that NCAM2 is important for the maintenance of synapses. We demonstrate that NCAM2 is an integral synaptic component associated with the key molecular elements of excitatory glutamatergic synapses. Knock-down of NCAM2 expression in cultured hippocampal neurons results in reduced formation of glutamatergic synapses. Surprisingly, over-expression of NCAM2 also affects numbers and maturation of glutamatergic synapses. Furthermore, analysis of neuronal activity using genetically encoded Ca2+ reporters shows that neurons over-expressing NCAM2 are hyperactive.
Our results thus indicate that NCAM2 is an important regulator of synapse formation and maintenance. Changes in expression of NCAM2 can cause abnormalities in synapse function and neuronal activity affecting information processing in the brain and contributing to intellectual disability.

Poster 341 – Tuesday 6th December

SIMULTANEOUS ALTERATIONS IN NUCLEOSOME POSITIONS AND DNA METHYLATION PREDICT EPIGENOME-WIDE CHANGES IN ALZHEIMER'S DISEASE.

Mr Andrew Phipps[2], Dr Adele Woodhouse[2], **Dr Phillippa Taberlay[1]**

[1]School of Medicine, University Of Tasmania, [2]Wicking Dementia Research and Education Centre, University of Tasmania

The molecular mechanisms underlying epigenetic alterations observed in Alzheimer's disease (AD) have not been established. Here, we take advantage of the APP/PS1 transgenic mouse model, which develop hallmark β-amyloid plaques and plaque-associated synapse loss, to establish the role of nucleosomes in epigenetic remodelling events corresponding to disease pathology. To do so, we perform nucleosome occupancy and methylation sequencing (NOMe-Seq), which uniquely enables concurrent investigation of DNA methylation, nucleosome positions and chromatin accessibility across a single strand of DNA from an individual cell. Nuclei were collected from the forebrains of 8-month old APP/PS1 mice (n=2) and age-matched wildtype mice (n=2) before enzymatic treatment with M.*CviPI* methyltransferase and bisulphite sequencing (n=20/genotype). We observed significant changes in nucleosome occupancy at gene regulatory regions of known risk factor genes (e.g. *TBXA2R*, *F2RL2* and *SORBS3*), including the modulation of nucleosome positions capable of physically altering transcriptional potential across gene promoters. Remodelling events at distal enhancer regions (e.g. *BIN1* super-enhancer) are also evident. Our findings suggest that the physical architecture of chromatin is remodeled, alongside DNA methylation, at gene regulatory elements and predict widespread epigenome-wide reprogramming events early in AD.

Poster 342 – Monday 5th December

IN VITRO AND IN VIVO PHYSIOLOGY OF LOW CONCENTRATIONS OF ZN2+ IN ARTIFICIAL CEREBROSPINAL FLUID

Haruna Tamano[1], Yukina Shakushi[1], Miku Sasaki[1], Atsushi Takeda[1]

[1]Department of Neurophysiology, School of Pharmaceutical Sciences, University of Shizuoka

Artificial cerebrospinal fluid (ACSF), i.e., extracellular medium, used for in vitro and in vivo experiments, includes Ca2+ and Mg2+, but not other divalent cations such as Zn2+. It has been recognized that intracellular Zn2+ signaling is critical for neuronal and glial functions. To clarify the physiological significance of extracellular Zn2+, the action of low nanomolar concentrations of Zn2+ in ACSF was examined in both in vitro and in vivo experiments. Spontaneous presynaptic activity assessed with FM 4-64 is significantly suppressed in the stratum lucidum of brain slices from young rats bathed in ACSF containing 10 nM ZnCl2, but not in 1 nM ZnCl2 and in 10 nM CuCl2 or 10 nM FeCl3, indicating that hippocampal presynaptic activity is enhanced in brain slices prepared with ACSF without Zn2+. The basal (static) levels of intracellular (cytosolic) Ca2+ are not significantly modified in brain slices from conventional ACSF without Zn2+. To examine the in vivo action of 10 nM ZnCl2 on long-term potentiation (LTP) induction, the recording region was perfused using a recording electrode attached to a microdialysis probe. The magnitude of LTP was not significantly modified by perfusion with ACSF containing 10 nM ZnCl2, compared to perfusion with ACSF without Zn2+, but attenuated by perfusion with ACSF containing 100 nM ZnCl2. The present study indicates that the basal levels of extracellular Zn2+, which may be low nanomolar concentrations, are linked to synaptic activity. The addition of a low nanomolar concentration of Zn2+ to ACSF may be critical for observing synaptic activity.

Poster 343 – Tuesday 6th December

ABSENCE OF SERPINB6 CAUSES SENSORINEURAL HEARING LOSS WITH MULTIPLE HISTOPATHOLOGIES IN THE MOUSE INNER EAR: NEW DEVELOPMENTS FOR NON-SYNDROMIC HEARING LOSS IN HUMANS

Dr Justin Tan[1], Dr Dion Kaiserman[2], Dr Monica Prakash[2], Professor Stephen O'Leary[1], Professor Philip Bird[2]

[1]The University Of Melbourne, [2]Monash University

Serpins are a large family of structurally related inhibitors of serine and cysteine proteases. The first case of a protease inhibitor associated with hearing loss in humans was reported in 2010 and this inhibitor was identified as SERPINB6 (Sirmaci et al. 2010). Humans with homozygous deficiency of SERPINB6 show hearing loss when they reach adulthood. Affected individuals noticed a progressive loss in hearing but no audiograms were performed to follow the extent of their hearing loss with age. It is unknown how SERPINB6 deficiency causes hearing loss and where it is expressed in the cochlea. We use mouse models to help us understand this deafness syndrome in humans by analysing mutant mice in which the orthologous Serpinb6a gene is replaced by enhanced green fluorescent protein. SERPINB6 is present in the neurosensory epithelium, lateral wall and spiral limbus of the cochlea, with highest levels in the inner and outer hair cells of the organ of Corti, cells lining the inner sulcus, and supporting cells distributed along the epithelial gap junction layer to the outer sulcus. Measurements of hearing thresholds in these mice demonstrated age-related hearing loss in all homozygous null, but not heterozygous, mice. The defect is associated with progressive cellular degeneration within the cochlea, beginning with the hair cells. The pattern of SERPINB6 expression in the human cochleae shares similarities with that in the mouse. SERPINB6 is a protease inhibitor that is essential for protecting cochlear cells from degeneration. Its deficiency causes progressive hearing loss in both humans and mice.
This work is supported by the National Health and Medical Research Council of Australia and the Garnett Passe and Rodney Williams Memorial Foundation.

Poster 344 – Monday 5th December

HABITUATION OF VAGAL RESPIRATORY SENSORY FEEDBACK

Mr Elliot Teo[1], Ms Tessa Borloo[1], Prof Peter Silburn[1], Dr Hari Subramanian[1]

[1]*Queensland Brain Institute, The University of Queensland, St Lucia*

Vagal sensory feedback is critical for higher brain centers to modulate basic respiratory rhythm in the context of survival (flight/fright/freezing) and/or emotional expression (vocalization, fear, stress/anxiety). However, vagal feedback is prone to habituation i.e. desensitization of respiratory phase prolongation, which would impact on respiratory switching mechanisms required for survival and emotional expression. How the vagus nerve habituates is virtually unknown and remains one of the most challenging questions in sensory-motor neuroscience. We stimulated the vagus nerve at various frequencies, voltages and time constants to examine habituation thresholds in urethane anethetised rats. We assessed the outcome of the vagus nerve habituation as 'efference copy' via measuring diaphragm function (EMG) during sustained stimulation. Our data shows that latency of habituation in vivo is frequency dependent but not voltage dependent. A range of frequency thresholds govern vagal habituation for various outcomes such as; 1) expiratory reflex prolongation, 2) generation of short-term apnoea and 3) generation of sustained apnoea. Vagal rebound during sustained stimulation also depended on the frequency of stimulation. We further examined the impact of vagal habituation on inspiratory, expiratory and post-inspiratory durations, shape and amplitude of the diaphragm (diaphragmatic effort) and hypoxia challenges. Our findings serve a benchmark for understanding vagal habituation principles and for designing vagal neuromodulation therapy for treatment of both emotional and respiratory disorders.

Poster 345 – Tuesday 6th December

A POPULATION OF NON-NEURONAL GFRALPHA-3 EXPRESSING CELLS IN THE BONE MARROW RESEMBLES NON-MYELINATING SCHWANN CELLS

Thai J[1]**,** Green A[2], Purton L[2], and Ivanusic J[1]

[1]Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, Australia. [2]Department of Medicine, St Vincent's Institute, The University of Melbourne, Fitzroy, Australia.

The bone marrow is innervated by sympathetic and sensory nerve terminals. We have identified a close association between nerve terminals and non-neuronal cells that express the artemin receptor (GFRα3) in the bone marrow microenvironment. The aim of the present study was to determine the identity of these cells using combinations of immunohistochemistry and fluorescent-activated cell sorting. High-resolution imaging of bone marrow sections revealed that the GFRα3$^+$ cells are in intimate contact with, but are clearly distinct from, nerve terminals. We used various combinations of markers to distinguish different cell types, including non-myelinating Schwann cells (GFAP, p75 NTR, nestin) and neural crest-derived mesenchymal stem cells (Sca-1, PDGFRα, nestin) and showed that the GFRα3$^+$ cells expressed markers of non-myelinating Schwann cells only. Further analysis of bone marrow sections from Wnt1-reporter mice demonstrated that the cells originate from the neural crest, as expected for non-myelinating Schwann cells, and real-time qPCR confirmed that the Wnt1$^+$ cells expressed GFRα3. 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. Thus the findings suggest a role for an interaction between nerve terminals and artemin/GFRα3 signaling in the development and/or regeneration of the peripheral nervous system, or perhaps in the maintenance of the hematopoietic niche in the bone marrow

Poster 346 – Monday 5th December

UNDERSTANDING ONE OF THE MOST ELABORATE EYES IN THE WORLD: INVESTIGATIONS INTO THE NEURAL ARCHITECTURE AND PROCESSING PATHWAYS OF THE STOMATOPOD (MANTIS SHRIMP) VISUAL SYSTEM.

Dr Hanne Thoen[1], Professor Nicholas Strausfeld[2], Professor Justin Marshall[1]

[1]*Queensland Brain Institute, University of Queensland,* [2]*Department of Neuroscience, University of Arizona*

With specialised photoreceptors detecting 12 different spectral channels in addition to linear and circular polarised light that result in a total of 20 information channels from the retina, stomatopods have a very elaborate visual system. The colour and circular polarisation receptors are located in an equatorial band called the midband resulting in a divided eye with an upper and lower hemisphere of achromatic, linear polarisation sensitive receptors. How stomatopods process the information from the various receptors is still unknown, although behavioural and neuroanatomical experiments suggest a they use a form of visual processing distinct from other animals. Here we present the first detailed insights into the stomatopod visual architecture, describing the visual pathway from the optic lobes to the central complex (CX). The information from the midband remains segregated from the hemispheral parts through the two first optic lobes, while laterally extending collaterals of midband relays intersect hemispheric presentations in the lobula, indicating channel integration in this area. Projecting from the lobula are subsets of neuronal relays terminating in distinct neuropils containing ensembles of micro and macroglomeruli. While these are thought to be homologues of the optic glomerular complex of insects and other crustaceans, the high number of glomeruli may be unique in stomatopods. Finally, we found that the stomatopod CX contained features such as a prominent protocerebral bridge a divided central body and paired noduli, making their CX more similar to insects than to other crustaceans, indicating either a close evolutionary relationship or an interesting example of convergent evolution.

Poster 347 – Tuesday 6th December

STROMAL INTERACTING MOLECULE 1 (STIM1) IS IMPORTANT FOR AXON GUIDANCE IN VIVO

Adrian Thompson[1], Dr Rob Gasperini[1], Dr Kaylene Young[2], Dr Lisa Foa[1]

[1]University of Tasmania, [2]Menzies Institute for Medical Research

Nervous system development requires axons to navigate to their synaptic targets, and calcium signaling is a key determinate of axon guidance. However, calcium regulatory mechanisms that control axon guidance remain to be fully understood. Store-operated calcium entry (SOCE) was recently identified as a source of calcium for axon guidance. SOCE is a form of calcium entry triggered by calcium depletion from the endoplasmic reticulum, a process regulated by the ER-resident calcium sensory protein stromal interacting molecule 1 (STIM1). We have previously demonstrated that STIM1 expression is necessary for axon guidance *in vitro*. However the importance of STIM1 for axon guidance *in vivo* is not well understood.
Utilizing the stereotypy of zebrafish motor neuron pathfinding, we investigated the importance of STIM1 expression for calcium signaling during axon guidance *in vivo*. STIM1 expression was shown to be important for correct axon guidance. STIM1 morphant axons exhibited a 14° rostral shift in the angle of axon outgrowth away from the horizontal myoseptum, which is a key checkpoint for motor neuron axon pathfinding. Furthermore, reduced STIM1 expression perturbed calcium signaling in pathfinding axons. Calcium transients were abolished in 24% of STIM1 morphant axons compared with 0% of control morphants. In STIM1 morphant axons at the horizontal myoseptum, the number of bursting events and the number high frequency spikes were decreased compared with control axons. These findings reveal that STIM1 expression is important for correct calcium signaling in pathfinding axons *in vivo*, suggesting that SOCE is a critical calcium regulatory mechanism for axon guidance.

Poster 348 – Monday 5th December

ROLE OF AXONAL CALCIUM IN KAINIC ACID-INDUCED AXON DEGENERATION IN CULTURED CORTICAL NEURONS

Nan Tian[1], Kelsey Hanson[1], Prof. James Vickers[1], Dr. Anna E King[1]

[1]Wicking Dementia Research And Education Centre

Calcium signalling has long been associated with axon degeneration caused by neurodegenerative diseases. However the role of axonal calcium alterations in axon degeneration induced by excitotoxicity is poorly understood. To investigate this, compartmented microfluidic chambers were used to selectively culture cortical axons isolated from the somatodendritic compartment in vitro. Kainic acid (KA) was added to the soma compartment to model exitotoxicity and induce axon degeneration. We monitored calcium dynamics in axonal compartment by live cell calcium imaging. A transient calcium wave was observed in the axonal compartment immediately after KA (100 µM) application, which was significantly inhibited by EDTA or BAPTA to remove extracellular or intracellular calcium respectively. Pharmacological Inhibition of axonal endoplasmic reticulum (ER) ryanodine receptor, IP3 channel or the mitochondrial permeability transition pore (mPTP) partially attenuated the calcium increase triggered by KA, indicating the orchestration of intracellular calcium channels. KA induced transient calcium influx was dose independent in a range of 25 µM to 100 µM and wasn't affected by microtubule stabilization. A prolonged gradually increase of intracellular calcium was observed within 1 h post KA stimulation. In cultured cortical neurons, chelating calcium for half an hour to abolish the early calcium influx in the axon after KA exposure only slightly delayed the progress of axon degeneration, suggesting that the early calcium signalling may not be the key component of KA induced axon degeneration program. Future studies will investigate the role of the secondary axonal calcium increase in excitotoxin induced axon degeneration.

Poster 349 – Tuesday 6th December

THE CONNEXIN-43 MIMETIC PEPTIDE, PEPTIDE5, REDUCES PAIN HYPERSENSITIVITY IN A MOUSE MODEL OF NEUROPATHIC PAIN

Mr Ryan Tonkin[1], Miss Chamini Perera[1], Mr Sam Duffy[1], Mr Preet Makker[1], Dr Justin Lees[1], Dr Simon O'Carroll[3], Professor Louise Nicholson[3], Professor Colin Green[3], Dr Catherine Gorrie[2], Dr Gila Moalem-Taylor[1]

[1]The University Of New South Wales, [2]University of Technology, [3]University of Auckland

Accumulating evidence points to a key role for spinal astrocytes in the pathogenesis of peripheral neuropathy. Astrogliosis is associated with the opening of undocked connexin (Cx) hemichannels and efflux of small excitatory molecules, such as ATP. An increased extracellular concentration of ATP causes assembly of the NLRP3 inflammasome, which activates caspase-1 and cleaves pro-inflammatory cytokines to their active form, thus contributing to neuroinflammation. Astrocytes express high levels of Cx43, and this is up-regulated in several animal models of neuropathic pain. In this study, we investigated the effects of blocking Cx43 hemichannels on neuropathic pain following a chronic constriction injury (CCI) of the sciatic nerve. We found that nerve-injured mice developed significant mechanical allodynia by day 7, and at day 10, Cx43 protein expression was doubled in the ipsilateral spinal cord. Eight hours following a spinal intrathecal injection of Peptide5 at day 10, Cx43 was significantly reduced and mechanical pain hypersensitivity was improved compared to saline injected CCI mice. We also found that NLRP3 protein expression was increased 3-fold in CCI mice and was reduced to naïve levels following Peptide5 treatment. Our findings demonstrate that spinal delivery of Peptide5, following nerve injury, is effective at targeting Cx43 hemichannels and reducing mechanical pain hypersensitivity. This reduction in neuropathic pain behaviours was associated with decreased NLRP3 suggesting that Cx43 hemichannel opening is linked to inflammasome activation in the spinal cord.

Poster 350 – Monday 5th December

IMAGING THE ENTERIC NERVOUS SYSTEM REVEALS EXTENSIVE FAST SYNAPTIC TRANSMISSION TO CGRP IMMUNOREACTIVE PUTATIVE INTRINSIC SENSORY NEURONS IN THE MOUSE COLON

Mr Lee Travis[1], Dr Tim Hibberd, Professor Marcello Costa, Professor Simon Brookes, Professor Nick Spencer

[1]Flinders University

CGRP is a reliable immunohistochemical marker for Dogiel Type 2 neurons in the myenteric plexus of mouse colon. When intracellular microlectrode recordings are made from these neurons fast synaptic inputs are not evoked by single pulse electrical stimuli, whereas other functional classes of myenteric neurons do receive prominent fast synaptic inputs under these conditions. We determined whether CGRP+ve neurons respond to single pulse electrical nerve stimuli when calcium imaging is used to record their activity. An electron multiplied-CCD camera and calcium indicator (Fluo-4) were used to record dynamic changes in calcium concentration in myenteric nerve cell bodies at 35°C. In response to single pulse transmural electrical stimuli (0.4ms duration, 70V), robust calcium transients were evoked by 167 out of 171 single pulses delivered to 43 CGRP+ve neurons in 5 animals (97% response rate). Calcium transients evoked by single pulse stimuli were abolished by hexamethonium (100μM) in 37 out of 40 trials (n=5). In 27 out of 43 CGRP+ve neurons (67%), ongoing spontaneous calcium transients were observed, which were also blocked by hexamethonium. During spontaneous colonic migrating motor complexes, two neurochemically distinct populations of neurons (nNOS+ve, neurons with small somas) and large (CGRP+ve neurons) became temporally phase-locked in their firing over periods sometimes exceeding 20s. Hexamethonium abolished synchronized firing of all myenteric neurons (n=5). The findings show that large CGRP+ve myenteric neurons in the mouse colon receive prominent inputs from fast synaptic pathways that utilize nicotinic receptors.

Poster 351 – Tuesday 6th December

SUPEROXIDE DISMUTASE-1; A POTENTIAL MEDIATOR OF NEURONAL DEGENERATION UNDER COPPER-DEFICIENT CONDITIONS IN THE PARKINSON'S DISEASE BRAIN?

Benjamin G Trist[1], Katherine M Davies[2], Veronica Cottam[1], Sian Genoud[1], Richard Ortega[3], Stéphane Roudeau[3], Asuncion Carmona[3], Kasun De Silva[2], Valerie Wasinger[4], Simon JG Lewis[5], Perminder Sachdev[6], Bradley Smith[7], Claire Troakes[7], Caroline Vance[7], Christopher Shaw[7], Glenda M Halliday[2], Dominic J Hare[8], Kay L Double[1]

1. Discipline of Biomedical Science and Brain and Mind Centre, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia. 2. Neuroscience Research Australia, Sydney, NSW 2031, Australia and the University of New South Wales, Sydney, NSW 2052, Australia. 3. University of Bordeaux, CENBG, UMR 5797, F-33170 Gradignan, France and CNRS, IN2P3, CENBG, UMR 5797, F-33170 Gradignan, France. 4. Bioanalytical Mass Spectrometry Facility, Mark Wainwright Analytical Centre, the University of New South Wales Australia, Kensington, NSW 2052, Australia. 5. Healthy Brain Ageing Program, University of Sydney, Sydney, NSW 2006, Australia and Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia. 6. The University of New South Wales, Sydney, NSW 2052, Australia and Prince of Wales Hospital, Randwick, NSW 2031, Australia. 7. Maurice Wohl Clinical Neuroscience Institute, King's College London, Camberwell, SE5 9NU, London, UK. 8. 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

Copper dyshomeostasis is central to neurodegenerative cascades in several neurodegenerative disorders and represents a tractable target for the development of disease-modifying therapies. We have shown that intraneuronal copper is selectively reduced (55-65%) in degenerating regions of the Parkinson's disease (PD) brain and in corresponding regions in Incidental Lewy body disease (pre-clinical PD), suggesting that copper deficiency is associated with early and progressive neuronal death in PD. We investigated changes in the copper-dependent neuronal antioxidant, superoxide dismutase 1 (SOD1), and demonstrated that SOD1 exhibits evidence of copper-deficiency and misfolding, as well as reduced dismutase activity, in degenerating regions of the PD brain. The co-localisation of aggregated SOD1, copper-deficiency and neuronal loss in the PD and Incidental Lewy body disease brain suggests that these mechanisms may be linked. Indeed SOD1 aggregates in the PD brain share numerous features with neurotoxic motor neuron SOD1 deposits in familial amyotrophic lateral sclerosis, which are believed to result from the mismetallation of SOD1 in these neurons. We suggest that the pathophysiological pathways resulting in SOD1 aggregation are similar in PD and amyotrophic lateral sclerosis and propose a role for SOD1 aggregation in neuronal death in PD. This shared proteopathy suggests potential for the translation of therapeutic approaches targeting SOD1 metal status, currently in clinical trials for amyotrophic lateral sclerosis, into a disease-modifying treatment for PD.

Poster 352 – Monday 5th December

EXCITATORY DRIVE OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS MAINTAINS HYPERTENSION BUT NOT BAROREFLEX DYSFUNCTION IN CHRONIC KIDNEY DISEASE

Underwood CF, Tallapragada VJ, Phillips JK, Hildreth CM.

Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia.

Hypertension and impaired baroreflex control of blood pressure contributes to the high risk of cardiovascular disease in chronic kidney disease (CKD). We tested the hypothesis that enhanced excitatory neurotransmission in the hypothalamic paraventricular nucleus (PVN),

an important neurohumoral regulatory area, contributes to hypertension and baroreflex dysfunction in the Lewis Polycystic Kidney rat (LPK) model of CKD. In urethane-anaesthetised LPK (n=6) and control Lewis rats (n=7) instrumented to record arterial pressure, heart rate (HR) and sympathetic nerve activity (SNA), inhibiting PVN neuronal activity with bilateral microinjection of muscimol (10mM) evoked a larger decrease in systolic blood pressure in LPK vs. control (P<0.01), reduced HR in LPK only and had no effect on renal or splanchnic SNA in either strain. In a separate cohort of LPK (n=7) and control (n=8) rats, PVN microinjection of the ionotropic glutamate receptor antagonist kynurenic acid (100mM) produced a greater depressor response in LPK vs. control (P<0.001) but was largely without effect on HR or renal SNA in either strain. Baroreflex curves for HR and SNA were rightward shifted in LPK and gain was reduced vs. Lewis (*P*<0.05). PVN microinjection of muscimol or kynurenic acid did not improve baroreflex function in either strain. These data suggest that in the LPK, elevated glutamatergic drive to the PVN contributes to hypertension, but not baroreflex dysfunction. Further investigation will be necessary to elucidate the source of this aberrant neuronal activity and the downstream mechanism (i.e. neuroendocrine or autonomic) responsible for mediating PVN-dependent hypertension in this model of CKD.

Poster 353 – Tuesday 6th December

LEARNING DEFICITS IN THE TAU58/2 MOUSE MODEL CORRESPOND TO DEFICITS IN LONG-TERM POTENTIATION FORMATION.

Miss Julia van der Hoven[1], Dr Annika Van Hummel[1,2], Dr Anne Harasta[1], Ms Magdalena Przybyla[1], Dr Yazi Diana Ke[2], Dr Janet Van Eersel[1], Prof. Lars Mattias Ittner[1,3,4]

[1]Dementia Research Unit, UNSW Australia, [2]Motor Neuron Disease Unit, UNSW Australia, [3]Transgenic Animal Unit, UNSW Australia, [4]Neuroscience Research Australia

Neuronal functional deficits and neurofibrillary tangle formation occur in Alzheimer's Disease (AD) and frontotemporal lobar degeneration (FTLD) in the hippocampus. The hippocampus is a brain region important for learning and memory. During learning, hippocampal neurons undergo synaptic plasticity with long-term potentiation (LTP). Behavioral and electrophysiological experiments were undertaken to establish whether the novel TAU58/2 transgenic mouse model, which mimics the histopathological features of AD and FTLD, presents with impairments in learning and memory formation due to functional deficits.
Tau58/2 male mice (2, 4 and 6 month-old) underwent learning/memory testing using Morris Watermaze (MWM). Electrophysiological analysis with theta-burst stimulation on brain slices from 1 and 4 month-old TAU58/2 mice were used to determine LTP in the hippocampus (Schaffer collateral pathway to CA1 region) compared to wildtype littermates.
The 4 month-old and 6 month-old Tau58/2 mice show significantly impaired learning/memory formation compared with wildtype littermates in the MWM (P<0.05). These mice take longer to find the platform at both ages and 6 month-old mice spend less time moving towards the platform (P<0.05). Furthermore, we found deficits in LTP formation observed in brain slices from the 4 month-old mice (P<0.01). Taken together, this data is indicative of progressive worsening of tau pathology and neuronal functional deficits seen in the TAU58/2 mouse model.
Learning/memory formation deficits are observed at the same age when decreased LTP formation was detected in the hippocampus of TAU58/2 mice.

Poster 354 – Monday 5th December

BINARY α1β3 GABAA RECEPTORS: A NOVEL TARGET FOR MOTOR RECOVERY IN STROKE.

Dr Petra Sonia Van Nieuwenhuijzen[1], Dr Ahmed Che Has[1], Dr Andrew Clarkson[2], Tristan Reekie[3], Dr Michael Kassiou[3], Dr Mary Chebib[1]
[1]The University Of Sydney, Faculty of Pharmacy, [2]Department of Anatomy, Brain Health Research Centre, The University of Otago , [3]School of Chemistry, The University of Sydney

Zolpidem is a non-benzodiazepine hypnotic with paradoxical effects; it can "awaken" people from a coma and improves speech and motor function in patients with severe brain injury. We recently identified the □1□3 $GABA_A$ receptor containing an □1-□1 interface as the target through which zolpidem can be distinguished from other benzodiazepines. This receptor is upregulated after stroke and non-sedative doses of zolpidem significantly improve motor function in stroked mice. To further our findings we investigated the cyclopyrrone eszopiclone and the imidazopyridine, DPA-713 for activity at binary α1β3 $GABA_A$ receptors. Receptors were expressed in *Xenopus* oocytes and the effects of the compounds were studied and compared to those at the □1□3□2 $GABA_A$ receptor that possesses the classical α1-γ2 benzodiazepine site using 2-electrode voltage clamp. Our results show that both eszopiclone as well as DPA-713 significantly enhance the GABA response at the binary □1□3 $GABA_A$ receptors containing an □1-□1 interface. Compounds that are able to modulate GABA at these binary receptors are potential therapeutics for motor recovery after brain injury and we aim to test these compounds in our stroke model.

Poster 355 – Tuesday 6th December

A NEW LOOK AT GENERAL ANAESTHESIA: UNCOVERING A SYNTAXIN1A-MEDIATED PRESYNAPTIC MECHANISM

Queensland Brain Institute Oressia Zalucki[1], Adekunle Bademosi[1,3], Michael Troup[1], Rachel Gormal[1,3], Benjamin Kottler[1], James Steeves[1], Shu Liu[1], Shanker Karunanithi[2], Victor Anggono[1,3], Frederic Meunier[1,3], Bruno van Swinderen[1]
[1]Queensland Brain Institute, The University of Queensland, [2]School of Medical Science, Griffith University, [3]Clem Jones Centre for Ageing Dementia Research

General anaesthetics such as propofol and isoflurane are sedatives that also produce a behaviourally inert state conducive to surgery. It is now understood that many general anaesthetics potentiate sleep pathways in the brain through GABAergic mechanisms, but it remains unclear what additional mechanisms achieve the profound loss of responsiveness that allows surgery to proceed. Using single-molecule imaging in mammalian neurosecretory cells and *Drosophila* synapses, we found that the mobility of membrane-bound syntaxin1A, a key SNARE protein, is significantly restricted by clinically relevant concentrations of propofol (3 μM). This presynaptic effect of propofol is associated with reduced synaptic efficacy, particularly for evoked neurotransmitter release. Co-expression of a truncated syntaxin1A protein lacking a transmembrane domain completely blocked these propofol effects. Co-expression of the same truncated syntaxin1A in *Drosophila* flies produced behavioural resistance to propofol as well as isoflurane. Anaesthetics appear to impair synaptic efficacy by interfering with syntaxin1A and SNAP-25 recruitment prior to SNARE complex assembly. Reduced synaptic efficacy across all synapses in the brain is likely to impair functional connectivity and thus be an important mechanism for general anaesthesia maintenance during surgery.

Poster 356 – Monday 5th December

ACUTE INFLAMMATION ORCHESTRATES MOTOR NEURON REGENERATION IN THE ZEBRAFISH SPINAL CORD

Ms Celia Vandestadt[1], Dr Timo Friedrich[1], Dr Sultan Alasmari[1], Prof Graham Lieschke[1], Dr Jan Kaslin[1]

[1]Australian Regenerative Medicine Institute

Human spinal cord injury results in a devastating loss of function and lifelong disability, in part due to a lack of regenerative potential. Zebrafish have a remarkable capacity to regenerate their central nervous system (CNS) following injury. The regenerative response of CNS cell types following injury is beginning to be unravelled, however the underlying molecular mechanisms initiating these responses are not yet fully understood. Here we apply pharmacological intervention to our larval spinal cord lesion assay, coupled with *in vivo* live confocal imaging, to identify cellular and molecular events underlying successful motor neuron regeneration in the zebrafish. Our results showed that larval zebrafish have rapid functional and morphological regeneration, which is accompanied by a classical inflammatory response that is rapidly resolved. Mutants lacking the hematopoietic cell lineage show significantly reduced motor neuron regeneration (34% reduction, $p<0.001$) and reduced functional recovery (12% reduction, $p<0.01$) compared to siblings. Glucocorticoid treatment reduces recruitment of both macrophages (70% reduction, $p<0.0001$) and neutrophils (60% reduction, $p<0.001$), which was also accompanied by a significant reduction in motor neuron regeneration (45% reduction, $p<0.0001$). Furthermore, we found that immune stimulation increases early motor neuron regeneration (18% increase, $p<0.05$) and partially rescues the poor regeneration of immune compromised mutants (9% increase, $p<0.05$). Collectively our results suggest that factors relating to acute immune response are required for successful regeneration of motor neurons following spinal cord injury in zebrafish.

Poster 357 – Tuesday 6th December

ADRENALINE SECRETION IS MEDIATED BY INTERACTIONS BETWEEN OREXIN AND GLUTAMATE IN THE VENTROLATERAL MEDULLA

Dr Willian Korim[1,2], Dr Mazher Mohammed[3], Dr Yoichiro Otsuka[3], Dr Simon McMullan[4], Dr Barbara Fam[1], Associate Professor Anthony Verberne[1]

[1]Department of Medicine, University of Melbourne, [2]Howard Florey Institute of Neuroscience and Mental Health, University of Melbourne, [3]Centre for Neuroscience, Department of Human Physiology, Flinders University, [4]Faculty of Medicine & Health Sciences, Macquarie University

The glucose counter-regulatory response to hypoglycaemia involves excitation of adrenal sympathetic premotor neurons resulting in adrenaline release. It is known that this response is partly mediated by excitatory inputs from orexin neurons, which also express glutamate. Whether orexin and glutamate interact in order to excite adrenal sympathetic premotor neurons is unknown. In anaesthetized male Ataxin-3 rats, in which orexin neurons are genetically ablated, we found that adrenaline and glucagon release in response to insulin-induced hypoglycaemia is reduced (both $P<0.05$) compared to wild type rats. Plasma levels of noradrenaline remained unchanged in response to insulin-induced hypoglycaemia. Additionally, glutamate receptor-induced activation of adrenal premotor neurons in the rostral ventrolateral medulla (RVLM) depends on the effects of orexin. Microinjection of glutamate into the RVLM, where adrenal premotor neurons are found, increased adrenal sympathetic nerve activity (ASNA)(~400 %). The increase in ASNA in response to activation of glutamate receptors in the RVLM was reduced following blockade of orexin type-1 and type-2 receptors ($P<0.05$). The results suggest that adrenaline release is a selective response to hypoglycaemia and involves an interaction between glutamate and orexin facilitating the activation of adrenal sympathetic premotor neurons in the RVLM.

Poster 358 – Monday 5th December

ENHANCED DIABETIC PHENOTYPE AND NEURODEGENERATION IN A NOVEL TRANSGENIC MOUSE MODEL WITH OVERLAPPING PANCREATIC AND CEREBRAL AMYLOID PATHOLOGIES.

Nadeeja Wijesekara[1], Roesmary Ahrens[1], K Ha[1], Miheer Sabale[2], Imran Khan[2], PaulE Fraser[1,2,3], **Giuseppe Verdile[2]**
[1]Tanz Centre for Research in Neurodegenerative Disesaes, University of Toronto, [2]School of Biomedical Sciences, Curtin University, [3]Department of Medical Biophysics

Alzheimer's disease (AD) risk is significantly elevated in individuals with type 2 diabetes (T2D) and insulin resistance is prevalent in AD patients. To better understand the interactions between these two chronic diseases, we developed a double transgenic mouse model (DTG), co-expressing human islet amyloid polypeptide (hIAPP) and human amyloid precursor protein (APP) with Swedish and Indiana mutations that enhances Aβ levels. The diabetic (fasting glucose, insulin and glucose tolerance) and neurodegenerative (cognition, synaptic activity, amyloid pathology) phenotypes were assed in these mice and compared to Non-Tg, APP, or mice expressing hIAPP only. The DTG mice show marked hyperglycemia and were glucose intolerant. Pancreatic islet amyloid deposition and reduced β-cell area was exacerbated in DTG mice, compared to hIAPP mice only, contributing to the exacerbated diabetic phenotype in these mice. APP mice showed signs of insulin resistance, as they were hyperinsulinemic but were normoglycemic. The DTG mice showed greater hippocampal A deposition and reduced hippocampal immunostaining of synaptophysin ($p<0.05$) and a trend towards greater impairments in learning and memory than APP mice. DTG and APP mice showed increased plasma A and reduced insulin signaling in brain, liver and skeletal muscle, suggesting that Aβ accumulation contributes to cerebral and peripheral insulin resistance. Aβ immunisation improved the diabetic phenotype observed in the DTG mice. Overall, this data indicates a role for A in both T2D and AD pathogenesis.

NW was supported by postdoctoral fellowships from the Canadian Institute of Health Research (CIHR) and the Canadian Diabetes Association and from CIHR (MOP-115056) to PEF. GV was supported by the Western Australian Department of Health, Merit Award. National Health and Medical Research Council (NHMRC) (APP1105698) was awarded to PEF and GV.

Poster 359 – Tuesday 6th December

INSULIN RESISTANCE IS ASSOCIATED WITH REDUCTIONS IN SPECIFIC COGNITIVE DOMAINS AND INCREASES IN CSF PTAU IN COGNITIVELY NORMAL CONTROLS.

Simon Laws[1,2,3], Scott Gaskin[1], Amy Woodfield[1], Velandai Sirkanth[4], David Bruce[5], Paul Fraser[1,6], Nadeeja Wijesekara[6], Samantha Burnham[7], Greg Savage[8], Vincent Dore[7], Colin Masters[9], Paul Maruff[9], Stephanie Rainey Smith[2], Christopher Rowe[9,10], Victor Villemagne[9,10], Ralph Martins[1,2,11], **Giuseppe Verdile[1,2]**

[1]School of Biomedical Sciences, Curtin University, [2]School of Medical Sciences, Edith Cowan University, [3]Co-operative Research Centre for Mental Health, [4]Department of Medicine, Monash University, [5]School of Medicine and Pharmacology, University of Western Australia, [6]Tanz Centre for Research in Nueordegnerative Diseases, University of Toronto, [7]CSIRO, [8]School of Psychology, Macquarie University, [9]The Florey Institute of Neuroscience and Mental Health, [10]Austin Health, [11]Departmemt of Biomedical Sicences

Animal studies show that inducing type 2 diabetes/insulin resistance can promote AD pathology. In human studies, the associations with cognition and AD pathological features as the disease progresses remain unclear. To begin to address this we investigated if changes in the indices of insulin resistance (IR) (HOMA-IR) or pancreatic β -cell function (HOMA-B) were associated with altered cognitive performance and pathological features of AD, in the Australian Imaging, Biomarker and Lifestyle (AIBL) Study. Differences in the calculated HOMA indices between clinical classifications were assessed using an ANCOVA via a General Linear Model approach, whilst associations with cognitive five composite measures (Global cognition, Verbal Episodic Memory, Visual Episodic Memory, Executive Function and Language) and pathological features (CSFAβ42, CSF tTau/pTau, hippocampal volume and neocortical amyloid burden) were investigated at baseline using linear regression analysis. HOMA-IR ($F=7.240$, $p=0.001$) was greater at baseline in those with MCI and AD compared with controls. Strongest associations were observed with HOMA-IR. Individuals with higher IR had significantly poorer composite scores in verbal episodic memory ($P=0.046$) and executive function ($P=0.009$), primarily driven by relationships within controls. Higher IR was associated with greater CSF total ($P<0.05$) and phosphorylated ($P<0.05$) Tau levels in controls. We show that greater HOMA-IR is associated, in cognitively normal older people, with poorer performance on measures of episodic memory, executive function whilst also presenting with greater CSF tau levels. Insulin resistance may be a strong early contributing factor in influencing cognitive decline.

Poster 360 – Monday 5th December

THE EFFECTS OF PRE- INTO POST-NATAL CIGARETTE SMOKE EXPOSURE ON NICOTINIC ACETYLCHOLINE RECEPTORS AND APOPTOTIC MARKERS IN THE MOUSE BRAINSTEM.

Miss Arunnjah Vivekanandarajah[1,2], Mr Yik Lung Chan[4], Dr Hui Chen[4], Dr Rita Machaalani[1,2,3]
[1]Department of Medicine, Blackburn Building, DO6 , [2]The BOSCH Institute, [3]The Children's Hospital, [4]School of Life Sciences, Faculty of Science, University of Technology Sydney

Infants exposed to pre-into post-natal cigarette smoke have increased respiratory and cardiac abnormalities and increased risk of Sudden Infant Death Syndrome (SIDS). Nicotine, the major neurotoxic component of cigarette smoke, induces its actions by binding to nicotinic acetylcholine receptors (nAChR), subsequently resulting in increased apoptosis. Using a maternal smoke exposure (SE) mouse model of pre- into post-natal SE exposure, we used immunohistochemistry to measure the expression of nAChR subunits α2, α3, α4, α5, α7, α9, β1 and β2, and two common markers of apoptosis: Caspase-3 and TUNEL in seven nuclei of the medulla and facial nucleus of the Pons in male mice. We compared pups of dams (n=5) exposed to two cigarettes (nicotine $\leq$ 1.2 mg, CO $\leq$ 15 mg) twice daily for six weeks prior to mating, during gestation and lactation with pups of sham (SHAM, n=5) dams exposed to air under the same condition. We found that there were predominant changes in the 3, 7, and 1 nAChR subunits and both Casp-3 and TUNEL protein in several nuclei of the medulla. The hypoglossal, dorsal motor nucleus of the vagus and the nucleus of the solitary tract were the nuclei showing greatest changes. This is the first study to demonstrate that nAChR subunits are affected following pre-into post-natal SE with simultaneous changes in apoptotic marker expression and that the nuclei affected are the ones with cardio and respiratory related functions.

Poster 361 – Tuesday 6th December

ADENOSINE RECEPTORS REGULATE SUSCEPTIBILITY TO ACOUSTIC INJURY

Dr Srdjan Vlajkovic[1], Mr Kaushi Ambepitiya[1], Dr Meagan Barclay[1], Dr Detlev Boison[2], Professor Gary Housley[3], Professor Peter Thorne[1]
[1]Department of Physiology and Centre for Brain Research, The University of Auckland, [2]RS Dow Neurobiology Laboratories, Legacy Research, [3]Department of Physiology and Translational Neuroscience Facility, School of Medical Sciences, UNSW

Our previous studies have shown that the stimulation of A_1 adenosine receptors in the inner ear can mitigate the loss of sensory hair cells and hearing loss caused by exposure to traumatic noise or ototoxic drugs. Here, we focus on the role of adenosine receptors (AR) in the development of noise-induced neural injury in the cochlea using A_1AR and A_{2A}AR null transgenic mouse lines. Wildtype and AR deficient mice were exposed to octave band noise (8-16 kHz, 100 dB SPL) for 2 hours to induce cochlear injury and hearing loss. Auditory thresholds and input/output functions were assessed using auditory brainstem responses (ABR) before and two weeks post-exposure. The loss of afferent synapses and spiral ganglion neurons (SGN) were assessed by quantitative histology. Young A_1AR null ($A_1AR^{-/-}$) mice (6-8 weeks old) displayed a high frequency hearing loss (ABR threshold shift and reduced ABR wave II amplitudes). This hearing loss was further aggravated by acute noise exposure and exceeded the hearing loss in the wildtype controls and $A_{2A}AR^{-/-}$ mice. All mice experienced the loss of synaptic ribbons and SGN after noise exposure, but the loss of SGN was significantly higher in $A_1AR^{-/-}$ mice than in the other two genotypes. The $A_{2A}AR^{-/-}$ had better preservation of afferent synapses and the minimal loss of SGN after noise exposure. The findings suggest that the loss of A_1AR expression results in an increased susceptibility to cochlear neural injury and hearing loss, whilst absence of A_{2A}AR increases cochlear resistance to acoustic trauma.

Poster 362 – Monday 5th December

LKB1 AND TAU PHOSPHORYLATION IN ALZHEIMER'S DISEASE

Volkerling A[1], Bi M[1], Delerue F[1 2], Ittner LM[1 2], Ittner AA[1].

[1.] Dementia Research Unit, Department of Anatomy, Faculty of Medicine, School of Medical Sciences, UNSW Australia, Sydney, NSW, 2052, Australia.
[2.] Transgenic Animal Unit, Mark Wainwright Analytical Centre, UNSW Australia, Sydney, NSW, 2052, Australia
[3.] Neuroscience Research Australia, Sydney, NSW, 2036, Australia

Alzheimer's disease (AD) and the frontotemporal lobar dementia subtype, FTLD-tau, are increasingly prevalent forms of dementia that are referred to as tauopathies. Pathologically, they are characterised by the presence of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau, as well as neuronal dysfunction due to defective brain metabolism. Liver Kinase B1 (LKB1) plays a vital role in regulating neuronal polarity via phosphorylation of microtubule associated proteins, including tau. LKB1 is also a crucial regulator in neuronal energy homeostasis via activating AMP-activated protein kinase, the master regulator of energy homeostasis. Interestingly, AMPK activation has been shown to be upregulated in AD neurons, preceding the aggregation of pathological tau and formation of NFTs. The molecular mechanisms linking tau pathology in AD and FTLD-tau to AMPK are unknown. Here, we addressed the molecular pathway interactions between tau pathology, energy metabolism (AMPK) via LKB1. Using *in vitro* cell culture and tissue analysis work, as well as analysing animal models (TAU58 mice) of AD and FTLD-tau generated by the Ittner Lab We found specific tau phosphorylation regulating LKB1 activity, reducing its function in disease. The LKB1/AMPK pathway may therefore serve as target to restore healthy brain metabolism in neurodegenerative diseases.

Poster 363 – Tuesday 6th December

REGIONAL AND TEMPORAL ANALYSIS OF BIOCHEMICAL ALTERATIONS IN A NEW TDP-43 MOUSE MODEL OF MOTOR NEURON DISEASE

Dr Prachi Mehta[1], Dr Christoph Krisp[2], Dr Mark Molloy[2], **Dr Adam Walker[1]**

[1]Department of Biomedical Sciences, [2]Australian Proteome Analysis Facility

Motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease caused by the loss of brain and spinal cord motor neurons. Almost all MND patients are characterised at autopsy by the presence of pathology containing the RNA/DNA-binding protein TDP-43. However, the causes and consequences of TDP-43 pathology development remain unclear. Newly developed transgenic TDP-43 mouse models expressing cytoplasmically-targeted TDP-43 in the brain and spinal cord (rNLS mice) develop, for the first time, both ALS-like TDP-43 pathology and disease phenotype. Using advanced quantitative sequential windowed data-independent acquisition of total high-resolution mass spectra (SWATH-MS) mass spectrometry, we profiled proteomic changes in brain and spinal cord of transgenic rNLS TDP-43 mice compared with litter-matched controls at pre-symptomatic, early-, mid- and late-symptomatic disease stages. Quantitative data were obtained from multiple peptides for >1500 proteins derived from soluble and insoluble protein fractions extracted following sonication and sequential ultra-high speed centrifugation. Of these, >60 proteins were detected with >1.5x statistically significant difference between transgenic rNLS TDP-43 mice and controls, covering novel proteins and those previously associated with MND, including the chaperone protein disulphide isomerase (PDI). Protein changes were validated using immunoblotting and immunofluorescence in neuronal cell culture, mouse brain and spinal cord and human ALS autopsy tissues, revealing a set of protein changes associated with disease onset and progression. These findings provide valuable insight into the early drivers of disease pathogenesis, and

highlight multiple new biochemical pathways for investigation of potential disease modifiers with the goal of developing new ALS therapeutics.

Poster 364 – Monday 5th December

THE ROLE OF NUCLEUS INCERTUS OREXIN RECEPTORS 1 AND 2 IN STRESS-INDUCED ALCOHOL SEEKING

Leigh Walker[1], Dr. Hanna Kastman[1], Dr Anna Blasiak[2], Marcin Siwiec[2], Elena Krstew[1], A/Prof. Andrew Gundlach[1], Prof. Andrew Lawrence[1]
[1]Florey Institute Of Neuroscience And Mental Health, [2]Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University

Alcohol use disorders are amongst the most common and devastating diseases in the world. Stress is a key precipitant of relapse in human alcoholics and in animal models of alcohol seeking. The brainstem *nucleus incertus* (NI) contains a population of relaxin-3 neurons that are highly responsive to psychological stressors; and the ascending NI relaxin-3/RXFP3 signaling system is implicated in stress-induced reinstatement of alcohol seeking. The NI receives orexin (OX) innervation and expresses OX_1 and OX_2 receptor mRNA. In alcohol-preferring (iP) rats, we examined the impact of yohimbine-induced reinstatement of alcohol seeking on OX neuronal activation, and the effect of bilateral injections into NI of the OX_1 receptor antagonist, SB-334867 (n = 16) or the OX_2 receptor antagonist, TCS-OX2-29 (n = 8) on stress-induced reinstatement of alcohol seeking. We also assessed the effects of OX-A on NI neuronal activity and the involvement of OX_1 and OX_2 receptors using whole cell patch-clamp recordings in rat brain slices. Yohimbine-induced reinstatement of alcohol seeking activated OX neurons in all OX-containing subregions of the hypothalamus. Bilateral NI injections of TCS-OX2-29 attenuated yohimbine-induced reinstatement of alcohol seeking. In contrast, intra-NI injection of SB-334867 had no significant effect. In line with these data, OX-A (600 nM) depolarized a majority of NI neurons recorded in coronal brain slices (18/28 cells), effects prevented by bath application of TCS-OX2-29 (10 μM), but not SB-334867 (10 μM). These data suggest an excitatory orexinergic input to NI contributes to yohimbine-induced reinstatement of alcohol seeking, predominantly via OX_2 receptor signalling.

Poster 365 – Tuesday 6th December

THE BDNF VAL66MET POLYMORPHISM MODERATES THE EFFECT OF COGNITIVE RESERVE ON 36-MONTH COGNITIVE CHANGE IN HEALTHY OLDER ADULTS

Dr David Ward[1], Dr Ross Andel[2], Associate Prof Mathew Summers[1,3], Prof James Vickers[1]
[1]Wicking Centre, University Of Tasmania, [2]School of Aging Studies, University of South Florida, [3]Mind and Neuroscience Institute, University of the Sunshine Coast

Both cognitive reserve (CR) and the *BDNF* Val66Met gene polymorphism are independently associated with rate of cognitive decline in preclinical Alzheimer's disease. Despite this, little is known regarding the potential interactive effects of CR and *BDNF* Val66Met on non-clinical older age cognitive function. This study was designed to investigate the consequences of variation in CR and *BDNF* Val66Met on 36-month cognitive change in 448 healthy older adults participating in the Tasmanian Healthy Brain Project (*M* age = 60.43, *SD* = 6.79). Trained assessors administered annual assessments of neuropsychological, health, and psychosocial function at baseline, 12-, 24-, and 36-month follow-up. A series of linear mixed effects models were fitted separately for each composite cognitive *Z* score (episodic memory, working memory, executive function, language processing), adjusted for age, gender, *APOE* genotype, and symptoms of depression and anxiety. Our main result was that, compared to *BDNF* Val homozygotes (estimate = -0.028), executive function trajectories of *BDNF* Met carriers (estimate = 0.125) were significantly more affected by baseline cognitive reserve (p = .011). Specifically, a comparison of trajectories for low vs. high CR groups revealed small differences in *BDNF* Val homozygotes (p = 0.050, Cohen's d = 0.311), yet medium-large differences in *BDNF* Met carriers (p = 0.007, Cohen's d = 0.646). Furthermore, a decline in performance over time was only identified in the low CR/*BDNF* Met group. Overall, this pattern of results suggests that *BDNF* Met confers a vulnerability to lower CR, leading to detrimental ageing-related changes in higher-order cognitive functions.

Poster 366 – Monday 5th December

EXPLORING THE EFFECTS OF AUTOPHAGY INDUCERS AND INHIBITORS IN A TRANSGENIC ZEBRAFISH MODEL OF SPINOCEREBELLAR ATAXIA TYPE-3

Maxinne Watchon[1,2,3], Kristy Yuan[2], Nickolce Mackovski[3], Dr Nicholas Cole[2], Professor Garth Nicholson[1,2,3], Dr Angela Laird[2]

[1]University Of Sydney, [2]Macquarie University, [3]ANZAC Research Institute

Spinocerebellar ataxia type-3 (SCA3), otherwise known as Machado-Joseph disease, is a fatal neurodegenerative disease affecting the coordination and control of the muscles in patients. This hereditary, autosomal dominant disease is caused by a trinucleotide repeat (CAG) region in the gene ATXN3/MJD1. The CAG trinucleotide encodes glutamine (Q) rich region within the protein ataxin-3. SCA3 patients polyQ regions than 40 repeats comparto healthy patients We have previously shown that our transgenic SCA3 zebrafish have motor as early as six days fertilization can be reversed by with a calpain inhibitor compound. This compound also reduced human ataxin-3 levels induction of the macroautophagy pathway. e explored the of various autophagy induc and inhibit that affect different parts of the mTOR-dependent and –independent macroautophagy pathway. different FDA approved drugs significant motor of these SCA3 zebafish a darkness escape response test. he levels of ataxin-3 . These results suggest

Poster 367 – Tuesday 6th December

HYPOXIA REGULATED MICRORNA-210 IN NEURONAL PLASTICITY

Miss Michelle Watts[1], Miss Sarah Williams[2], Professor Charles Claudianos[3]

[1]Queensland Brain Institute, University Of Queensland, [2]Diamantina Institute, The University of Queensland, [3]Monash Institute of Cognitive and Clinical Neuroscience, Monash University

The hypoxia-regulated microRNA-210 (miR-210) is a highly conserved miRNA, known to regulate metabolism and cell cycle under hypoxic conditions. Recently in our lab we found that miR-210 is also involved in honeybee learning and memory, raising the question of how neural activity may induce hypoxia regulated genes and in turn how miR-210 may regulate plasticity not only in insects but also in more complex mammalian systems. Using a biotin pull-down approach in the human-derived SH-SY5Y neuroblastoma cell system we have experimentally identified 1077 unique target genes of miR-210 by RNAseq. Of note among these targets there was a significant enrichment of neurodegenerative KEGG pathways including Alzheimer's, Huntington's and Parkinson's disease. Among pulled-down target genes we also identified a number of genes known to be regulated by neuronal activity and with significant neural-plasticity functions. Using dual-luciferase assays we have validated that miR-210 directly interacts and down-regulates genes including the NMDA-R subunit GRINA, the translation initiation binding protein EIF4EBP1 and the beta-actin isoform, ACTB. This was a significant finding as actin dynamics are crucial to neuronal plasticity and evidence from our lab suggests miR-210 may target the homologous, actin 5C (*Act5C*) in insects, indicating a potentially conserved regulatory pathway of miR-210. Additionally we have shown that miR-210 overexpression results in significant morphological changes in neuronal-like differentiated SH-SY5Y cells, including decreased neurite length and neurite branching. This data suggests a potentially novel mechanism of how innately occurring metabolic changes may couple plasticity to neuronal activity through hypoxia-regulated genes such as miR-210.

Poster 368 – Monday 5th December

C-REACTIVE PROTEIN AS A MARKER OF INFLAMMATION IN ACUTE PSYCHOSIS AND SCHIZOPHRENIA

Dr Thomas Weickert[1,2,3], Isabella Jacomb[2], Dr Clive Stanton[4], Rohini Vasudevan[4], Hugh Powell[4], Dr Dennis Liu[5], Dr Cherrie Galletly[5], Dr Rhoshel Lenroot[1,2,3,4], Dr Maryanne O'Donnell[1,4], Dr Cynthia Weickert[1,2,3]

[1]School of Psychiatry, University Of New South Wales, [2]Neuroscience Research Australia, [3]Schizophrenia Research Institute, [4]Prince Of Wales Hospital, [5]University of Adelaide

There is increasing evidence for the role of the inflammation in schizophrenia. Immune alterations have been shown in subgroups of patients with schizophrenia. C-reactive protein (CRP) is an acute phase reactant protein mainly produced by hepatocytes in response to an increase in circulating pro-inflammatory cytokines. Recent meta-analyses have reported a high prevalence of elevated CRP in schizophrenia which has been associated with acute psychosis and impaired cognition in schizophrenia. Here, we examine the prevalence of CRP as a marker of inflammation in two independent samples of individuals with psychosis: 1) individuals with acute psychosis and 2) chronically ill people with a diagnosis of schizophrenia. Elevated CRP levels were defined as ≥ 3mg/L. In the acutely ill sample, CRP levels were significantly elevated (60% having a CRP level above normal). Individuals with acute psychosis also displayed significantly increased neutrophil-to-lymphocyte ratio (NLR) levels and a significantly higher proportion (67%) had positive anti-nuclear antibodies. In acutely ill patients with psychosis CRP and NLR levels remained consistently high at repeated admissions. In the chronically ill patients with schizophrenia, CRP levels were significantly elevated compared to healthy controls, with 44% of chronically ill patients displaying clinically elevated CRP levels. The elevated CRP group of chronically ill patients displayed significantly worse current IQ, working memory, and attention/processing speed. Taken together, the present findings support previous findings suggesting that inflammatory markers decrease with resolution of acute psychotic symptoms and further support the use of adjunctive anti-inflammatory treatments in schizophrenia.

Poster 369 – Tuesday 6th December

CXCR4 EXPRESSION INCREASED THROUGHOUT ADULTHOOD IN THE HUMAN SUBEPENDYMAL ZONE

Ms Christin Weissleder[1,2,3], Dr Mari Kondo[1,2,3], Dr Maree Webster[4], Prof Cynthia Shannon Weickert[1,2,3]

[1]Schizophrenia Research Laboratory, Neuroscience Research Australia, [2]Schizophrenia Research Institute , [3]School of Psychiatry, Faculty of Medicine, University of New South Wales, [4]Laboratory of Brain Research, Stanley Medical Research Institute

Signalling of the chemokine CXCL12 through its receptors, CXCR4 and CXCR7, has been shown to regulate progenitor proliferation, neuronal differentiation and migration; however, little is known about their temporal expression profile in the human subependymal zone (SEZ, also subventricular zone), where neurogenesis continues into adult life. In this study, we examined by quantitative polymerase chain reaction CXCR4 and CXCR7 expression in the human SEZ from young adulthood into aging (n=50, 21-103 years) and ascertained whether these changes correlate with indices of proliferation and neuronal differentiation. We found that CXCR4 mRNA significantly increased with age in the SEZ ($r=0.50$, $p<0.0001$), whilst CXCR7 mRNA remained stable ($r=0.07$, $p=0.63$). Cell proliferation marker transcripts neither correlated with CXCR4 ($r=-0.004$, $p=0.98$) nor with CXCR7 mRNAs ($r=0.12$, $p=0.42$). Neuronal progenitor marker expression positively correlated with CXCR4 ($r=0.46$, $p=0.001$) and CXCR7 mRNAs ($r=0.55$, $p<0.0001$), whereas immature neuron marker transcripts negatively correlated with CXCR4 mRNA ($r=-0.33$, $p=0.01$) and reached trend levels of statistical significance for CXCR7 mRNA ($r=-0.27$, $p=0.056$). Our findings indicate that CXCR4 and CXCR7 are continuously expressed in the adult SEZ, with an age-related increase of CXCR4 transcripts. Further studies are

required to anatomically map chemokine receptor expression to specific cell types and determine the regulatory role of CXCL12 in adult neurogenesis across the human lifespan. Understanding how altered chemokine signalling may impact the neurogenic potential of the SEZ will support studies examining the functional roles of adult neurogenesis, and also aid in the development of therapeutic strategies, where neuronal replacement may be beneficial.

Poster 370 – Monday 5th December

ANTIPSYCHOTIC-LIKE ACTION OF COMBINED ADMINISTRATION OF MGLU4 AND M4 RECEPTORS ACTIVATORS.

Wierońska JM[1], Woźniak M[1], Cieślik P[1], Acher F[2], Pilc A[1].

1.Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland. 2. Université Paris Descartes, Paris, France.

Both mGlu4 and M4 receptors were shown to be excellent target for antipsychotic-drug discovery. Activators of those receptors, both agonists and positive allosteric modulators (PAMs) were shown to possess excellent antipsychotic-like activity in animal studies. In the present study we show that mGlu4-M4 receptors may play in tandem, as the concomitant administration of the ligands of those receptors in subtreshold doses induced clear antipsychotic-like effects in chosen animal models of schizophrenia. MK-801-induced hyperactivity, DOI-induced head twitches, social interactions and novel object recognition tests were used. MK-801 (0.3 mg/kg) was administered as psychotomimetic agent. Drugs, LSP4-2022, mGlu4 agonist and VU152100, M4 PAM were administered with subtreshold doses. LSP4-2022 at the dose of 0.1 mg/kg and VU152100 0.5-2 mg/kg. When drugs were dosed separately they induced no any effect on animals, but when given together a clear antipsychotic-like effect was observed. Statistical analysis were calculated with two-way ANOVA, with $P<0.05$ as statistical significant. Our results show that the simultaneous administration of mGlu4-M4 activators is a good target for antipsychotic therapy.

The work is supported by the grant no 2015/17/B/NZ7/02984 from National Science Center in Poland to Joanna Wierońska

Poster 371 – Tuesday 6th December

EFFECTS OF DIFFERENT ANAESTHETIC AGENTS ON NERVE EXCITABILITY IN THE RAT TIBIAL NERVE

Wild BM[1], Arnold R[1], Mohan R[1], Morris R[1]

1Translational Neuroscience Facility, School of Medical Sciences, UNSW Medicine, UNSW, Sydney, Australia.

Nerve excitability testing (NET) is a reproducible and reliable means of testing axonal ion channel function and membrane potential in a clinical setting. However, these measures are indirect and are often challenging to interpret in a clinical setting. The use of these techniques in parallel with molecular studies in rodent models of disease provides an attractive translational methodology. However, different anaesthetic agents induce various alterations to the biophysical properties of peripheral nerves and thus nerve excitability results. Systematically examining these effects is important for the implementation of this technique as animals need to be unconscious, unlike that in humans. NET was performed on 12 adult Long Evans rats anaesthetized with either isoflurane through inhalation or subcutaneous injection of a 1:1 mixture of Hypnorm/Midazolam. Comparing the tibial nerve excitability results obtained under different anaesthetic agents revealed multiple significant differences in multiple parameters. In regards to stimulus-response parameters; rheobase and stimulus required to elicit a 50% response significantly greater in Hypnorm/Midazolam compared to isoflurane ($p<0.001$). Importantly, the Hypnorm/Midazolam group showed a significantly greater threshold change in the hyperpolarizing threshold electrotonus parameter when compared with the isoflurane group ($p<0.01$). Correspondingly, the slope of the current-threshold relationship ($p<0.01$) was greater in the Hypnorm/Midazolam group. These parameters both utilize long hyperpolarizing currents assessing inwardly rectifying properties of the axons and thus, taken together suggests enhanced inward recification under Isoflurane. In conclusion, this study demonstrates that different anaesthetic agents have different effects on motor excitability properties and should be considered before performing NET in animal models.

Poster 372 – Monday 5th December

ROSTRO-CAUDAL GRADIENT OF THE DENDRITIC INTEGRATIVE PROPERTIES OF LAYER 5 PYRAMIDAL NEURONS ACROSS THE PRIMARY VISUAL CORTEX

Mr Lee Fletcher[1], Professor Stephen Williams[1]

[1]Queensland Brain Instutute

The thickness of the neocortex varies over the neocortical mantle to determine the size of the dendritic arbor of pyramidal neurons. In order to preserve canonical cell-class properties across the neocortex, the electrical architecture of the dendritic arbor must parallel such morphological changes. The conservation of the integrative properties of a defined class of neuron with dendritic size has, however, not been directly explored. Here we use high-resolution anatomical reconstruction, multi-site somato-dendritic electrophysiological recordings and computational modelling approaches to demonstrate the physical size, electrotonic architecture, and mode of dendritic integration of layer 5B pyramidal neurons vary as a gradient across the rostro-caudal axis of the rat primary visual cortex (V1). Apical dendrite size linearly decreased from rostral to caudal V1 (soma-to-apical tuft path length: rostral= 893±14□m, Bregma=-6.3±0.1; caudal=768±20□m, Bregma=-

8.4±0.1; slope=-53□m/mm, p<0.0001), accompanied by increased efficacy of subthreshold voltage transfer between the base of the apical dendritic tuft and soma (caudal= 0.34±0.03; rostral= 0.12±0.01; n=42; p<0.0001). These results were maintained when resting voltage and ligand gated channels were blocked pharmacologically (caudal= 0.54±0.02; rostral= 0.44±0.02; n=42; p<0.004), to reveal unique electrotonic architectures when directly modelled. Consistent with this, multi-site recording revealed that rostral but not caudal neurons operated as compartmentalized integrators of dendritic excitatory input, by the generation of robust threshold dendritic electrogenesis. Our findings reveal that the integrative capacity of layer 5B pyramidal neurons transforms from multi-compartment, layered computations, to compact axo-somatic integration across V1. These data challenge the view that neocortical neuronal populations carry out canonical computations.

Poster 373 – Tuesday 6th December

PASSIVE NORMALIZATION OF DENDRITIC LIGHT SIGNALLING IN ALPHA RETINAL GANGLION CELLS

Dr Arne Brombas[1], Dr Simon Kalita-de Croft[1], Professor Stephen Williams[1]

[1]Queensland Brain Instutute

Dendritic spike generation has been shown to play an instrumental role in driving light-evoked action potential firing in direction-selective retinal ganglion cells (RGCs). To test if active dendritic integration is a universal signalling mechanism in the output neurons of the retina we made simultaneous somato-dendritic whole-cell recordings from identified rabbit OFF-alpha RGCs maintained ex vivo. Direct recordings revealed that light-evoked action potential firing of OFF-alpha RGCs was driven at the termination of receptive field spanning spot or annular light stimuli, because of the generation of powerful dendritic excitation. In contrast to recordings made from direction-selective RGCs, however, dendritic responses in OFF-alpha RGCs were not crowned by sodium channel mediated dendritic spikes, but were instead decorated by small amplitude back-propagating actions potentials. Indeed pooled data revealed the weak back-propagation of somatic current-evoked action potentials, which heavily attenuated with dendritic recording distance (50% attenuation=108□m; n=60). Furthermore, the injection of steps of positive current at dendritic sites failed to evoke dendritic electrogenesis from resting membrane potential. Despite this, spatially restricted light stimuli generated at sites widely distributed throughout the receptive field of OFF-alpha RGCs evoked action potential output, or somatic depolarization, with near equal efficacy. A normalization of the somatic impact of dendritic input also apparent when dendritic voltage responses were generated by steps of negative current, despite the electrically distributed nature of the dendritic arbor (dendrite to soma 50% voltage transfer=93□m, n=34). These data suggest that the wide-field responsiveness of OFF-alpha RGCs is mediated by the passive normalisation of dendritic input.

Poster 374 – Monday 5th December

NEUROINFLAMMATORY RESPONSE IN THE GROWTH RESTRICTED NEONATE

Dr Julie Wixey[1], Camille Muller[1], Dr Kirat Chand[1], Dr Susan Sullivan[1], Prof Paul Colditz[1], Dr Tracey Bjorkman[1]

[1]The University Of Queensland

Intrauterine growth restriction (IUGR) is a major cause of perinatal morbidity and mortality. The fetal brain is particularly vulnerable to the effects of IUGR which can increase risk of long-term neurological disorders. Few studies have focused on brain injury mechanisms in the growth restricted neonate, especially in pre-clinical animal models. Inflammation is associated with neuronal injury during acute hypoxic events but whether inflammation is associated with chronic hypoxic events such as IUGR remains to be clearly established. We hypothesize inflammation is prevalent in the IUGR brain and is associated with neuronal injury and glial cell disruption. Term newborn control and IUGR piglets were euthanized on day of birth. IUGR piglets demonstrated an increase in a number of pro-inflammatory cytokines in the parietal cortex in comparison to controls using qPCR Qiagen RT2 arrays. CSF-1 showed a 7-fold increase and CXCL10 a 56-fold increase. A decrease in anti-inflammatory cytokines were also evident with IL-10 and IL-4 demonstrating a 3- and 7-fold decrease respectively. Immunohistochemistry showed a significant increase in microglia (Iba-1) and astrocytes (GFAP) in the parietal cortex of IUGR piglets with concomitant oligodendrocyte disruption (CNPase) in the white matter. Our results demonstrate inflammation and glial cell disturbances in the IUGR piglet brain. The morphology of glial cells transform to a reactive state following damage to the central nervous system. There they release large amounts of pro-inflammatory cytokines perpetuating detrimental effects on neurons. Future studies will determine whether therapeutically targeting inflammation can prevent neuronal injury and improve neurological outcome in these infants.

Poster 375 – Tuesday 6th December

INCREASED INTRINSIC EXCITABILITY OF CORTICAL PYRAMIDAL NEURONS IN HEALTHY AGING

Dr Adele Woodhouse[1], Ms Kimberley Stuart[1], Professor James Vickers[1]

[1]Wicking Dementia Research and Education Centre, University of Tasmania

During the course of healthy aging cognitive function and memory both decline in association with changes in the morphology and electrical properties of pyramidal neurons in the hippocampus and cortex. Across a lifetime neurons regulate their intrinsic excitability to maintain a reliable transfer of information between cells while allowing for the synaptic plasticity that underlies learning and memory. Changes in intrinsic excitability have been correlated with cognitive function during aging, yet we lack a complete understanding of how intrinsic

excitability alters across the course of healthy aging. In the present work, we study the changes in biophysical properties of cortical pyramidal neurons in normal aging. Whole-cell patch-clamp recordings were performed in layer 2/3 cortical pyramidal neurons (from the frontal association area and M2) in adult 6 month-old (n=11) and aged 22 month-old (n=12) C57/Bl6 mice that had completed cognitive testing (Y maze, Barnes maze). Passive membrane properties differed between adult and aged cortical pyramidal neurons, with aged neurons exhibiting a higher input resistance and membrane time constant ($p<0.05$). There was a significant increase in intrinsic excitability in aged pyramidal neurons compared to adult pyramidal neurons ($p<0.05$). However, there was no difference between aged and adult cortical neurons in the slow afterhyperpolarization or the hyperpolarization induced sag (indicative of I_H). These data indicate that a biologically significant alteration occurs in the excitability of cortical pyramidal neurons in healthy aging.

Poster 376 – Monday 5th December

SELECTIVELY TARGETING THE P75 NEUROTROPHIN RECEPTOR (P75NTR): A NEW THERAPEUTIC STRATEGY IN PERIPHERAL DEMYELINATING DISEASES

Dr David Gonsalvez[1], Dr Agnes Wong[1], Dr Giang Tran[2], Mr Mithraka De Silva[1], Miss Rhiannon Wood[1], Dr Jessica Fletcher[1], Dr Suzanne Hodgkinson[2], Ms Lauren Giuffrida[1], A/Prof Tony Hughes[1], Prof Trevor Kilpatrick[1], Dr Simon Murray[1], **Dr Junhua Xiao[1]**

[1]The University of Melbourne, [2]The University of New South Wales

Current therapies for peripheral demyelinating diseases such as Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are anti-inflammatory to provide short-term benefits. However, neurological symptoms often persist and are underpinned by persisting demyelination. We have recently identified that the neurotrophin BDNF promotes peripheral nerve myelination via p75NTR expressed by neurons. This has led to the development of a cyclic pentapeptide (cyclo-$_D$PAKKR), which structurally mimics the putative $p75^{NTR}$-binding motif of BDNF. We now seek to determine if cyclo-$_D$PAKKR, acting via the $p75^{NTR}$, limits demyelination and promotes remyelination to produce a clinical benefit *in vivo* in experimental autoimmune neuritis (EAN), an animal model of CIDP. We found systemic administration of cyclo-$_D$PAKKR ameliorates EAN disease severity at a dose-dependent manner. Animals treated with cyclo-$_D$PAKKR over the disease peak displayed a significantly faster recovery profile compared to vehicle controls. These ameliorated clinical symptoms are supported by attenuated neurohistological deficits. We found administration of cyclo-$_D$PAKKR significantly reduced the extent of demyelination and protected against axonal degeneration, which was accompanied by significantly less inflammatory cell infiltration in peripheral nerves (the cauda equina and sciatic nerves) compared to vehicle treated animals, suggesting that cyclo-$_D$PAKKR exerts neuroprotective effects to ameliorate EAN. Importantly, cyclo-$_D$PAKKR failed to ameliorate EAN disease induced in p75NTR heterozygous mice compared to the WT control, suggesting a p75NTR-mediated beneficial effect. Together, our data demonstrate that cyclo-$_D$PAKKR ameliorates clinical and neurological deficits in EAN, an effect depending on p75NTR expression. Our findings suggest that selective targeting p75NTR, via cyclo-$_D$PAKKR, is potentially a novel therapeutic strategy to treat human demyelinating peripheral neuropathies such as CIDP.

Poster 377 – Tuesday 6th December

NEUROCHEMICAL CHARACTERISTICS OF MURINE LUMBOSACRAL DORSAL ROOT GANGLIA IN RESPONSE TO INFLAMMATION OF VAGINA

Yap P[1], Ji E[1], Sharma H[1], Vilimas P[1], Kyloh M[2], Spencer NJ[2], Barry CM[1] and Haberberger RV[1]

[1]Anatomy and Histology, [2]Human Physiology, Centre for Neuroscience, Flinders University of South Australia, Adelaide, Australia

Vulvodynia is a common chronic pain disorder of unknown aetiology. Neurochemical changes in dorsal root ganglia (DRG), including altered expression of nociceptor-associated markers such as calcitonin gene-related peptide (CGRP), isolectin-B4 (IB4) and the receptor transient receptor potential vanilloid 1 (TRPV1), are implicated in some chronic pain conditions. It is unknown if vulvovaginal inflammation induces similar changes. Therefore, this project characterised DRG associated with the vagina in female C57Bl/6 mice (6-8 weeks old) and identified changes following vaginal inflammation. Neuronal tracing of dextran-biotin injected into L5-S2 DRG confirmed distribution of DRG fibres in the vagina. Vaginal inflammation was induced by microinjection of complete Freund's adjuvant (CFA) in the distal vaginal wall, once (n=7) or twice (n=3) with 7 days between. Control animals (n = 4 per group) received saline injection. Multiple-labelling immunohistochemistry labelled L5 to S2 DRG neurons expressing the markers IB4, TRPV1 and CGRP. Confocal laser-scanning microscopy and subsequent image analysis identified neurons expressing one or more marker, and neurons were classified according to soma size. Biotin-containing nerve fibres were found more frequently in the distal than proximal vagina. Analysis of vaginal tissue confirmed the presence of inflammation at 7 and 14 days. At 14 days S1 DRG showed more neurons immunoreactive for IB4 without CGRP or TRPV1 compared to controls (ANOVA $p<0.01$). These neurons had small soma size. Findings indicate vaginal inflammation may induce neurochemical changes in associated DRG neurons. Further studies are required to determine if these changes may impact on DRG functions such as nociception

Poster 378 – Monday 5th December

REPEATED MILD TRAUMATIC BRAIN INJURY IN FEMALE RATS INCREASES LIPID PEROXIDATION IN NEURONS

Dr Nathanael Yates[1], Mr Stephen Lydiard[1], Ms Brooke Fehily[1], Ms Carole Bartlett[1], Assoc/Prof Melinda Fitzgerald[1]

[1]Experimental and Regenerative Neuroscience, School of Animal Biology, The University Of Western Australia

Repeated mild traumatic brain injuries (mTBI) are suffered by athletes participating in high impact sports, and may lead to long term and poorly understood deficits in cognitive and neurological functioning. Acute effects of repeated mTBI on cognition, oxidative stress, cellular dynamics and blood brain barrier (BBB) integrity were investigated using a closed-head mTBI model in adult female PVG rats. Anaesthetised animals received sham, 1x, 2x or 3x mTBI administered over 3 days with a weight drop device directly above the posterior skull (lambda), once per day. Consistent with the mildness of the injury, no gross neuromotor deficits were observed. However, subtle deficits in spatial memory were revealed only in the 2x mTBI group tested on day 4, using the Morris Water Maze ($p \leq 0.05$) and increased numbers cortical neurons positive for the lipid peroxidation marker 4-hydroxynonenal ($p \leq 0.05$). There were no other changes in indicators of oxidative stress, microglia/macrophage or astrocyte numbers, node/paranode structure nor evidence of cell death ($p > 0.05$). No changes for 3x mTBI on day 4 may be due to the reduced time since the last and recognised biphasic degeneration. Intravenous administration of Evans Blue dye revealed that the BBB is intact following posterior 3x mTBI at day 4, but not with anterior impacts (bregma) which have increased areas of Evans Blue extravasation ($p \leq 0.05$). Taken together, the results indicate that oxidative stress in neurons occurs acutely following repeated mTBI, associated with mild cognitive deficit, even in the absence of BBB disruption.

Poster 379 – Tuesday 6th December

POTENTIAL FUNCTION OF MIRNA-124 IN HUMAN NEUROGENESIS UNDER NORMAL AND INFLAMMATORY CONDITIONS

Dr. Ling Ye[1], Dr. Yunlong Huang[2], Dr. Jialin Zheng[1,2]

[1]Tongji University, [2]University of Nebraska Medical Center

Neurogenesis is the process by which neural progenitor cells (NPCs) differentiate into neurons that successfully incorporate into the neuronal circuitry of the CNS. The creation of new neurons is influenced by many factors including miRNAs. Exclusively expressed in the brain, miRNA-124 functions to reduce astrocyte differentiation, and promotes neurite outgrowth, and it is required for maintenance of mature neurons.
We hypothesize that miRNA-124 expression increases during the differentiation of human NPCs (hNPCs) into neurons, and expression of miRNA-124 is attenuated under neuroinflammatory conditions, resulting in increased levels of gliogenesis, a phenomenon associated with CNS injury.
Our previous miRNA microarray data have established that the expression of miRNA-124 increased gradually during the differentiation process of hNPCs. However, no big difference between the hNPCs and the differentiated cells at 1 day post-differrentiation, and miRNA-124 is significantly upregulated at 7 days post-differentiation into neurons. Our Real-time PCR data confirmed the miRNA microarray data. Then, we investigated the expression of miRNA-124 under inflammatory conditions mediated by IL-1β and/or TNF-α. We found that the stimulation of IL-1β and/or TNF-α increased the gliogenesis by upregulating the expression of mRNA and protein of glial fibrillary acidic protein (GFAP) ($p<0.01$), and decrease the neurogenesis by inhibiting the mRNA of microtubule associated protein-2 (MAP-2) and the protein of β-III-tubulin. In addition, genes regulated by miRNA-124 are also being assessed using software, and confirmed using Real-time PCR and Western blotting. Elucidating these genes are important in understanding the mechanism(s) by which changes in neurogenesis, due to miRNA-124 expression. We checked the expression of Signal transducer and activator of transcription 3 (STAT3) and sex determining region Y-box 9 (SOX9), and found that cytokine significantly increased the expression of these target genes ($p<0.05$). Furthermore, we knockdowned miRNA-124 by LNA (locked nuclear acid), and overexpressed miRNA-124 by lentivirus, and then checked the target genes of miRNA-124.
In conclusion, we confirmed that miRNA-124 is associated with neurogenesis in hNPCs, and it is downregulated under inflammatory conditions, which are caused by IL-1β and/or TNF-α. Cytokines stimulation promoted the astrogliogenesis and inhibited the neurogenesis. At the same time, the downstream target genes of miRNA-124 (STAT3 and SOX9) are upregulated by cytokines treatment. Overexpression of miRNA-124 decreases the expression of mRNA and protein levels of STAT3 and SOX9.

Poster 380 – Monday 5th December

TREATMENT WITH A CELL CYCLE INHIBITOR FOLLOWING PHOTOTHROMBOTIC STROKE INDUCES DIFFERENTIAL CHANGES TO RECOVERY IN MOTOR FUNCTION TESTS

Mr Wai Ping Yew[1], Ms Natalia Djukic[1], Professor Richard Woodman[1], Dr Hakan Muyderman[1], Professor Neil Sims[1]

[1]Flinders University

Altered connectivity between neurons in peri-infarct tissue following stroke is an important contributor to spontaneous recovery and a target for restorative therapies to improve outcomes. Responses of astrocytes and other glia in peri-infarct tissue influence the capacity for these adaptive responses. Treatment with olomoucine, a cell cycle inhibitor, has been shown previously to reduce reactive astrogliosis and restrict development of the glial scar (Wang et al, J. Clin. Neurosci. 15, 278-285, 2008) but the consequences for recovery were not assessed. The aim of the present study was to test whether recovery of motor function is altered by olomoucine administered after a stroke that was induced by photothrombosis and targeted the forelimb motor cortex in rats. Olomoucine (5 mg/kg) or vehicle was injected intraperitoneally at 1 hour and 24 hours after stroke. Rats showed severe impairment following the stroke in a skilled single pellet retrieval task and a test of the forelimb placing reflex induced by vibrissae stimulation. These functions recovered partially during the next four weeks. Recovery was significantly improved ($p<0.05$ for the interaction between treatment and time) for the olomoucine group as assessed based on total success or first retrieval success. In contrast, recovery of placing reflex was impaired in the olomoucine-treated group. The results point to complex responses to olomoucine that have different consequences depending on the aspects of motor function that are

evaluated. The findings also have potential implications for choosing tests to assess other restorative therapies. Supported by the NHMRC and Flinders Medical Centre Foundation.

Poster 381 – Tuesday 6th December

DISTINCT REACTIVATION OF MOVEMENT AND STILL EXPERIENCES ACROSS THE HIPPOCAMPAL-CORTICAL NETWORK

Jai Yu[1], Kenneth Kay[1], Marielena Sosa[1], Jason Chung[1], Matthias Karlsson[1], Margaret Larkin[1], Irene Grossrubatscher[4], Daniel Liu[1], Adrianna Loback[5], Loren Frank[1,2,3]

[1]University Of California San Francisco, [2]Howard Hughes Medical Institute, [3]Kavli Institute for Fundamental Neuroscience, [4]University of California Berkeley, [5]Princeton University

The brain encodes information about ongoing events in our experience that is subsequently stored in memory, for which the underlying neural mechanisms remain unknown. Experience takes place during movement between places and when being immobile in a single place. Hippocampal place cells maintain spatial representations of ongoing experience during both movement and immobility. These representations are later transiently reinstated during Sharp wave-ripples (SWRs), which are hypothesized to support the storage and retrieval of memory. However, only representations corresponding to movement through space between places have been identified during SWR across the hippocampus and cortex. If or how representations of being immobile at a single location are maintained in memory remain unknown. To answer these questions, we recorded neural activity simultaneously in the hippocampus and prefrontal cortex as rats performed spatial tasks that involve alternating bouts of movement and immobility. We found experiences during these periods of contrasting locomotor patterns are associated with distinct neural representations that are coherent across both regions. We then identified a subset of SWRs that correspond to representations of being immobile at a single place, which is distinct from those that correspond to movement-associated representations. Concurrently in PFC, individual cells also distinguish between these different reactivation events, showing time-locked excitation or inhibition in activity that is dependent on the content of hippocampal SWR reactivations. Our results suggest the brain encodes experiences during movement and immobility using distinct representations that are subsequently maintained as distinct memories across multiple regions.

Poster 382 – Monday 5th December

ZYMOSAN-INDUCED CYSTITIS IN THE GUINEA PIGS AS A MODEL OF INTERSTITIAL CYSTITIS.

Mrs Sarah Nicholas[1], Ms Lauren Keightley[1], Prof Simon Brookes[1], Prof Nick Spencer[1], **Dr Vlad Zagorodnyuk[1]**

[1]Flinders University

Cystitis is one of the commonest bladder disorders characterised by lower urinary tract symptoms such as pain, severe urgency and frequency. We aim to establish an animal model of interstitial cystitis. For this, anaesthetised guinea pigs were treated with protamine sulfate (10 mg/ml) followed by zymosan (10 mg/ml). Conscious voiding cystometry, viscreromotor responses (VMRs) in vivo and activity of pelvic afferent nerves in vitro were compared in controls and zymosan-induced cystitis. Hematoxylin and eosin staining consistently revealed mild infiltration of polymorphonuclear neutrophils in the bladder wall, 24 hrs after zymosan challenge. Compared to controls, guinea pigs with zymosan-induced cystitis have reduced voiding volume per void (by 30%) and inter-micturition interval (by 20%) and increased duration of micturition contractions (by 30%, n=5, $P<0.05$) observed during continuous conscious cystometry. Administration of 0.5 M KCl during continuous cystometry in anesthetized animals evoked a significant decrease (by 40%, n=4, $P<0.05$) in the inter-micturition interval compared to controls. Acute zymosan exposure induced hyperalgesia in the guinea pigs, measured as an increase in VMRs evoked by 20-60 mmHg bladder distensions (by 80% at 60 mmHg, n=6, $P<0.005$). In the presence of nicardipine (4 μM), the stretch-induced firing of pelvic low threshold stretch-sensitive afferents was unaffected in zymosan-induced cystitis, although the proportion of afferents responding to capsaicin (3 μM), was increased (by 40%, n=7, $P<0.05$). The data indicate that zymosan-induced cystitis is adequate animal model for interstitial cystitis since it evokes mild inflammation, hyperreflexia and hypersensitivity, similar to patients diagnosed with this debilitating bladder disorder

Poster 383 – Tuesday 6th December

NFIX REGULATES NEUROBLAST PROLIFERATION IN THE ADULT SUBVENTRICULAR ZONE

Dr Oressia Zalucki[1,2], Mr Lachlan Harris[1], Prof Richard Gronostajski[3], Assoc Prof Michael Piper[1,2]

[1]School Of Biomedical Sciences, The University of Queensland, [2]Queensland Brain Institute, The University of Queensland, [3]Department of Biochemistry, State University of New York

Within the adult brain, neural stem cells (NSC) reside within the hippocampus and subventricular zone (SVZ), retaining their division capabilities, existing mostly in a quiescent state. Whilst the importance of these NSC is appreciated for various functional requirements, such as learning and memory, the molecular regulators of adult NSC remain incompletely understood. We investigated the role of the nuclear factor I (NFI) transcription factor protein, NFIX, in adult NSC biology within the SVZ, given this protein is highly expressed in quiescent NSC. We show using two inducible knockout strains of NFIX that this transcription factor is a key mediator of cell proliferation within the SVZ. In particular, neuroblast proliferation and accumulation is significantly increased upon deletion of NFIX. Loss of NFIX is associated with a transient increase in NSC numbers, but NFIX is not required to maintain the quiescent state of these NSC. Our results are the first to

directly investigate the role of NFIX in adult neurogenesis within the SVZ, showing that this transcription factor is important for regulating neuroblast proliferation and accumulation.

Poster 384 – Monday 5th December

DEVELOPMENT OF A RAPID FUNCTIONAL ASSAY THAT PREDICTS GLUT1 DISEASE SEVERITY

Ms Sasha Maria Zaman[1,2], Dr Saul Mullen[1,3], Dr Elena Gazina[1], Dr A.Marie Phillips[1,5], Dr Snezana Maljevic[1], Dr Michael Hilderbrand[2,3], Dr John Damiano[2,3], Professor Holger Lerche[4], Dr Yvonne Weber[4], Professor Samuel Berkovic[3], Professor Ingrid.E Scheffer[1,3,6], Associate Professor Christopher Alan Reid[1], Professor Steven Petrou[1,2]

[1]The Florey Institute Of Neuroscience And Mental Health, [2]Department of Medicine (RMH) University of Melbourne, , [3]Department of Medicine (Austin Health), University of Melbourne, [4]4Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research,University of Tübingen , [5]5School of Biosciences, University of Melbourne, [6]Department of Paediatrics, University of Melbourne, Royal Children's Hospital,

Objective: GLUT1 (Glucose Transporter Type 1)-deficiency, due to mutations in *SLC2A1*, is associated with a wide range of epilepsies. One possible mechanism for this clinical heterogeneity is the variable extent to which mutations impact GLUT1 function. To test this possibility, we measured glucose transport by GLUT1 variants identified in patients with mild to severe epilepsies, developing a simple assay of GLUT1 function that is predictive of disease severity.
Methods: GLUT1 variants were assigned to three groups. The control group consisted of the NCBI's reference sequence and four population missense variants chosen from control databases. Nine variants associated with epilepsies or movement disorders, with normal intellect in all individuals, formed the mild group. Finally, the severe variants group included five missense mutations associated with classical, early onset GLUT1-encephalopathy. GLUT1 variants were expressed in *Xenopus laevis* oocytes and glucose uptake was measured to determine kinetics (V_{max}) and affinity (K_m) for each variant.
Results: Full kinetic analysis revealed that disease severity inversely correlated with rate of glucose transport between control (V_{max}= 28±5), mild (V_{max}=16±3) and severe (V_{max}=3±1) groups respectively. Affinities of glucose binding in control (Km =55±18) and mild (Km=43±10) groups were not significantly different. In the severe group, the binding affinity could not be accurately determined because of low transport rates. Kinetic analysis of glucose transport rate at one concentration (100 mM) was equally effective at separating the three groups.
Interpretation: This study shows that disease severity can be readily explained by the extent of GLUT1 dysfunction. This simple *Xenopus* oocyte assay complements genetic and clinical assessments that include cerebrospinal fluid glucose levels and red blood cell glucose uptake. In prenatal diagnosis this simple assay would be particularly useful as standard clinical assessments are not available.

Poster 385 – Tuesday 6th December

SUPPRESSOR OF CYTOKINE SIGNALLING 2 (SOCS2) INTERACTS WITH TROPOMYOSIN RELATED KINASE-B (TRKB) RECEPTOR AND REGULATES NEURONAL OUTGROWTH

Ms Akram Zamani[1], Mr Simon Murray[1], Mrs Ann M. Turnley[1]

[1]University Of Melbourne

TrkB, in response to its ligand Brain Derived Neurotrophic Factor (BDNF), can initiate signalling pathways leading to neuronal survival and neurite outgrowth. SOCS2, a negative regulator of the signalling, affects neurite outgrowth by regulating EGF and NGF receptor signalling. In this study we hypothesised that SOCS2 may also have a regulatory role in neuronal function by regulating TrkB. SOCS2 contain a central Src homology domain (SH2) which binds to phosphotyrosine residues a SOCS box domain, which targets proteins for ubiquitination and degradation. Full length and mutated forms of both TrkB and SOCS2 were constructed and transfected into HEK 293T cells to determine the regions of each protein required for interaction. These experiments SOCS2 interacts strongly with TrkB, but weakly with TrkB mutants lacking the juxtamembrane or kinase domain. involvement of the SOCS2 SH2 domain and the Kinase domain of TrkB in forming their interactionBoth SOCS2 and TrkB are abundant in the hippocampal region of the brain.Finally, neurite outgrowth was used as an indicator to examine the functional role of this interaction. Wildtype rat hippocampal neurons were transfected with SOCS2 mutant constructs, in the presence and absence of BDNF.Deletion of both the SH2 and the BOX domain in SOCS2 reduced neurite outgrowth in the absense of BDNF. Collectively, these data suggest that SOCS2 interacts with TrkB and this interaction regulates the neurite outgrowth of hippocampal neurons.

Poster 386 – Monday 5th December

CONTROLLING CALCINEURIN AND CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II WITH LIGHT.

Ms Agnieszka Zbela[1,2], Mrs Elise Devenish[1,2], Dr David Gell[1], Dr Robert Gasperini[1], Assoc Prof Lisa Foa[1], Dr John Lin[1]

[1]University Of Tasmania, [2]These authors contributed equally to this work.

Calcineurin (CaN) and calcium/calmodulin-dependent protein kinase II (CaMKII) are important for neuronal plasticity, in learning and memory as well as axon guidance in nervous system development. Valuable findings regarding CaN and CaMKII activity in plasticity have been obtained with genetic, pharmaceutical and electrophysiological methods.

Using optogenetic techniques, the activity of proteins can be controlled by exposure to light. This may enable precise spatiotemporal manipulation of CaN and CaMKII activity in individual cells, to investigate how these proteins are involved in neuronal plasticity. In this project, several photoactivatable recombinant proteins are being developed to manipulate CaN and CaMKII activity.
In one approach, inhibitory peptides have been fused to the blue light responsive LOV-Jα domain in order to prevent them from binding to their targets in the dark state and to reveal them upon light exposure. The VIVIT peptide is an inhibitor of CaN, whereas the CaMKIIntide peptide disrupts CaMKII signalling. A transcriptional reporter of CaN activity has been used to show that when fused to LOV-Jα VIVIT inhibits CaN, but that inhibition occurs under both light and dark conditions. A mammalian two-hybrid transcriptional assay has shown that binding between CaMKII and CaMKIIntide that is fused to LOV-Jα can be modulated in response to light, although the light-induced change is small. Ongoing work in design of fusion proteins is focused on increasing the difference between the dark and light state activity.
Ultimately, these novel optogenetic tools may provide control of CaN and CaMKII in order to investigate neuronal development and plasticity.

Poster 387 – Tuesday 6th December

PAIRWISE AND POPULATION DYNAMICS OF CORRELATED SPIKING ACTIVITY IN THE DORSAL LATERAL GENICULATE NUCLEUS OF MARMOSET

Natalie Zeater[1,2,3], Brandon R Munn[2,4], Samuel G Solomon[5], Bogdan Dreher[3], Soon K Cheong[1,3], Pulin Gong[2,4], Paul R Martin[1,2,3]

[1]Save Sight Institute, The University of Sydney, [2]ARC Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]School of Medical Sciences, The University of Sydney, [4]School of Physics, The University of Sydney, [5]Department of Experimental Psychology, University College London

Objective: At long (multi-minute) time scales, maintained or "spontaneous" neural activity in visual cortices of primates is characterised by: 1) on average, low correlation between pairs of simultaneously recorded cells (Smith et al., J Neurophysiol, 2013), and 2) diverse levels of coupling between local neuronal populations - ranging from highly correlated "choristers" to weakly correlated "soloists" (Okun et al., Nature, 2012). Our objective was to examine the correlations between pairs of neurons recorded from the principal dorsal thalamic visual centre - the dorsal lateral geniculate nucleus (dLGN). **Method**: Pairs and ensembles of visually-responsive dLGN cells were recorded in sufentanil-anaesthetised marmosets using a Neuronexus (16x2) silicon array probe. Pairwise correlations and spike-triggered population rates (stPRs) were calculated. Visual stimulus was a uniform grey 20 deg diameter field, of intensity ~50 cd/m^2. **Results:** As in the case of pairs recorded from the visual cortex, pairwise correlations at long timescales were, on average, low (0.04±0.08, n = 192 pairs), with amplitudes following a Gaussian distribution. There was no difference in the average correlation between homologous parvocellular (0.01±0.02), magnocellular (0.05±0.05) and koniocellular (0.05±0.09) pairs. At short (sub-second) timescales however, pairs showed dynamic correlation patterns ranging from strongly correlated to strongly anti-correlated. Calculation of stPRs shows the same range of population coupling reported for visual cortex. **Conclusion:** Pairwise and population properties of correlations in a synaptically weakly interconnected sub-cortical structure - dLGN are very similar to those in a synaptically strongly interconnected structure - the visual cortex.

Poster 388 – Monday 5th December

ANATOMICAL AND FUNCTIONAL CHARACTERISATION OF THE BRAIN RELAXIN-3/RXFP3 SYSTEM IN MICE: FOCUS ON HYPOTHALAMIC CIRCUITS

Cary Zhang[1,2], Dinara Baimoukhametova[3], Caitlin E Singleton[1,4], Mouna Haidar[1,2], Berenice E Chua[1], Annie Yang[1], Sherie Ma[1,2], Craig M Smith[1,2,5], Jaideep S Bains[3], Andrew L Gundlach[1,2,4]

[1]The Florey Institute Of Neuroscience And Mental Health, [2]Florey Department of Neuroscience and Mental Health, [3]Hotchkiss Brain Institute, University of Calgary, [4]Department of Anatomy and Neuroscience, The University of Melbourne, [5]School of Medicine, Deakin University

Mice *and* rats are routinely used in research on biological functions relevant to human health/disease, such as stress, anxiety and appetite; and our recent studies have revealed anatomical and functional similarities *and* differences between their brain relaxin-3/RXFP3 (peptide/GPCR) signalling systems. For example, immunohistochemical studies revealed GABA/relaxin-3 neurons in rat NI express the NGF receptor, TrkA, while in mouse NI TrkA immunoreactivity is observed in non-relaxin-3 neurons. In behavioural studies, similar to rats, mice centrally administered the selective RXFP3 agonist, RXFP3-A2 (1 nmol, 10-15 mice/group), displayed significantly reduced levels of the anxiety-like behaviours (p=0.002) pharmacologically-induced by the anxiogenic drug, FG-7142 (30 mg/kg, i.p.). Altered hypothalamic-pituitary-adrenal (HPA) axis activity may underlie such regulatory effects, but central administration of relaxin-3 in rats 'activates' corticotropin-releasing factor (CRF) neurons in the paraventricular hypothalamic nucleus (PVN). However, whole-cell patch-clamp recordings of CRF neurons in PVN of Crh-IRES-Cre:Ai14 mice revealed a significant reduction in spontaneous EPSC frequency (7 neurons, p=0.014) following 'local' application of RXFP3-A2; revealing an RXFP3-mediated inhibition of excitatory presynaptic inputs to these PVN CRF neurons. However, no change in HPA axis activity (reflected by serum corticosterone) was observed after icv or intra-PVN injection of RXFP3-A2 (1 and 0.1 nmol, 9-13 mice/group), consistent with the weak relaxin-3 innervation of, and RXFP3 levels in, mouse parvocellular PVN.
Our studies identify the relaxin-3/RXFP3 system as a possible target for treatment of psychiatric disorders, but highlight the need to evaluate preclinical pharmacological effects in experimental animals that are most relevant to non-human primate and human neurobiology and neuropathology.

Poster 389 – Tuesday 6th December

LOSS OF THE SULFATE TRANSPORTER SLC13A4 ALTERS BEHAVIOR AND NEUROGENESIS IN ADULT MICE

Ms Zhe Zhang[1], Dr. Michael Piper[1], Dr. Paul Dawson[1,2], Dr. David Simmons[1]

[1]School of Biomedical Science, The University Of Queensland, [2]Mater Research Institute

In the brain, sulfonation is a critical biochemical process that regulates the functions of extracellular matrix molecules and neurotransmitters. SLC13A4 is a sodium-dependent sulfate transporter primarily expressed in the placenta but also by the choroid plexus of the brain, which when genetically deleted causes severe fetal abnormalities and death in mice. Interestingly, *Slc13a4*$^{+/-}$ mothers lose a significant number of pups (~50%) shortly after birth, regardless of pup genotype, suggesting that *Slc13a4*$^{+/-}$ female mice may have deficits in maternal behaviors. In a test of maternal care behavior, *Slc13a4*$^{+/-}$ mothers took significantly longer to retrieve their pups when compared to *Slc13a4*$^{+/+}$ mothers. Furthermore, non-pregnant *Slc13a4*$^{+/-}$ mice had increased exploring behavior in a novel environment compared with *Slc13a4*$^{+/+}$ mice, indicating that the altered behavioral phenotypes are not necessarily pregnancy specific. Analysis of *Slc13a4* mRNA expression in choroid plexus throughout pregnancy identified a significant peak at gestational day 8.5, consistent with a peak in adult neurogenesis in the maternal subventricular zone (SVZ). This coincidence suggested that SLC13A4 may be important in regulating adult neurogenesis. We observed that *Slc13a4*$^{+/-}$ mice displayed significantly increased cell proliferation in the SVZ and subgranular zone (SGZ), although the additional cells born in these niches did not appear to integrate into their appropriate networks. Moreover, *Slc13a4*$^{+/-}$ mice exhibited less neurogenesis by one year of age, suggesting an early depletion of the neural stem cell niche. Our recent work indicates that altered sulfate transport within the choroid plexus has important implications for both behavior and the regulation of adult neurogenesis.

Poster 390 – Monday 5th December

DELAYED POST-CONDITIONING USING HIGH ALTITUDE TRAINING INDUCES NEUROPROTECTIVE ACTIONS AND ALLEVIATES COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF PHOTOTHROMBOTIC STROKE

Zidan Zhao[1]

[1]University Of Newcastle

Inspired from high altitude training in athletes, delayed hypoxic post-conditioning has been suggested as a potential non-pharmacological therapeutic intervention to enhance physiological functions in both healthy and diseased individuals. In the current study, we investigated whether high altitude training following stroke could improve brain repair. Undertaken in male C57B6 mice, we examined how exposure to an intermittent (8h/day) or consistent (24h/day) hypoxic environment (11% oxygen) for two weeks, following photothrombotic stroke, altered the infarct size, neuron loss and vascular remodelling of primary injury site in somatosensory cortex, as well as in the thalamic secondary neurodegeneration site. The results from our study revealed that exposure to hypoxic condition (both 8h and 24h/day for two weeks), induced smaller infarct volume and less neuron loss, and promoted vessel growth both in the infarct and thalamic site. Further, we observed that exposure to the hypoxic conditions improved stroked animals' performance in a computer-automated touchscreen-learning task. Collectively, these results suggest that high altitude training following photothrombotic stroke promotes brain recovery and could be considered as a candidate for clinical evaluation.

Poster 391 – Tuesday 6th December

THE INDIRECT EFFECT OF EPHA4 ON NEURONAL PRECURSOR PROLIFERATION IN THE ADULT HIPPOCAMPUS THROUGH D-SERINE MODULATION OF NMDA RECEPTOR

Dr Jing Zhao[1], Dr Dhanisha J Jhaveri[1,2], Dr Andrew W Boyd[3], Professor Perry F Bartlett[1]

[1]Queensland Brain Institute, The University of Queensland, [2]Mater Research Institute, [3]Queensland Institute of Medical Research

EphA4 has been shown to play various roles in the nervous system, however, the role of EphA4 in adult hippocampal neurogenesis is unknown. We observed that EphA4 knockout (EphA4$^{-/-}$) mice had increased numbers of 5-bromo-2'-deoxyuridine^{+}/doublecortin^{+} newborn neurons in the dentate gyrus. A similar increase was seen when wild-type mice were intra-hippocampally administered with an EphA4 antagonist, EphA4-Fc. In line with this, increased number of neurosphere were generated from precursors from EphA4$^{-/-}$ and kinase-dead mice in which only EphA4 reverse signalling remains intact. Application of EphA4 antagonists, 1E8 or EphA4-Fc, also enhanced precursor activation by inhibiting respectively either bidirectional signalling or forward signalling. These results indicate that inhibition of EphA4 forward signalling increased the precursor activation. However, this action of EphA4 on precursors was indirect, as flow cytometry analysis showed that precursors did not express EphA4, and EphA4-Fc did not activate clonal cultures of precursors. As the loss of EphA4 has been reported to decrease the D-serine in the hippocampus, we showed that supplementation of D-serine returned the increased proliferation induced by EphA4-Fc to control levels. Similarly, intraperitoneal injection of D-serine brought the increased proliferation in EphA4$^{-/-}$ mice back to wild-type levels. These results indicate that blocking EphA4 regulates precursor activation by modulating the release/synthesis of D-serine. Since D-serine is a co-agonist of *N*-methyl-D-aspartate receptors (NMDARs), we showed that blocking NMDARs mimicked the effects seen with EphA4$^{-/-}$ mice. Together, these findings indicate the novel role of EphA4 in the adult hippocampal proliferation, and demonstrate that D-serine-modulated NMDAR activation mediates this regulation.

Poster 392 – Monday 5th December

IMPROVED DIFFERENTIATION, NUCLEAR ENVELOPE MORPHOLOGY AND REDUCED ALPHA-SYNUCLEIN IN LRRK2 G2019S NEURAL CELLS TREATED WITH LRRK2 KINASE INHIBITORS

Miss Ye ZHAO[1,2], Lauren Schramko[1,2], Gayathri Perera[1], Professor Glenda Halliday[1,2], Dr Nicolas Dzamko[1,2]

[1]Neuroscience Research Australia, [2]School of Medical Sciences, University of NSW

The activating G2019S mutation in leucine-rich repeat kinase 2 (LRRK2) is a relatively common cause of inherited Parkinson's disease (PD). We therefore studied neural stem cells (NSC) from PD patient fibroblasts with the LRRK2 G2019S mutation (two clones from a single G2019S cell line) compared with control NSCs (two different cell lines) to identify effects of the kinase activating LRRK2 G2019S mutation and determine whether two different selective LRRK2 kinase inhibitors can reverse the mutation effects. At early passage, nestin-immunopositive NSCs were similar between lines, however both G2019S cell lines exhibited abnormal nuclear morphology revealed by lamin b1 immunostaining. This phenotype was exacerbated by prolonged **passage of NSCs, but improved with LRRK2 inhibition. We next examined the neuronal differentiation potential of control and G2019S NSC. After 7 days, G2019S cells were still nestin immunopositive, with some co-expression of the neuronal marker MAP2, compared to control cell lines, which were negative for nestin immunofluorescence and mainly immunoreactive for MAP2. Beta-3 tubulin confirmed reduced neurite extension in G2109S cells compared with controls. After 30 days, both G2019S cell lines, but neither control cell lines, exhibited accumulation of alpha-synuclein. In both G2019S neuronal cell lines, LRRK2 kinase inhibitors improved neural differentiation, increased neurite extension, improved the nuclear architecture of differentiated neurons and decreased alpha-synuclein accumulation. These results identify a variety of cellular defects in LRRK2 G2019S mutation expressing neurons, which can be reversed with LRRK2 kinase inhibitors.**

Poster 393 – Tuesday 6th December

PROTOCADHERIN 15 REGULATES OLIGODENDROCYTE PROGENITOR CELL PROLIFERATION

Miss Yilan Zhen[1], Dr Robert Gasperini[1,2], Dr Carlie Cullen[1], Dr Kaylene Young[1]

[1]The Menzies Institute For Medical Research,University of Tasmania, [2]The Menzies Institute for Medical Research and the School of Medicine, University of Tasmania

Oligodendrocyte progenitor cells (OPCs) are an immature cell population that proliferates and generates myelinating oligodendrocytes in the developing and mature central nervous system (CNS). OPCs also produce new myelinating oligodendrocytes in response to demyelinating injuries, however our ability to target these cells therapeutically is hampered as the signaling pathways that regulate OPC proliferation and oligodendrocyte maturation are poorly understood. Microarray and RNA sequencing data have identified a number of genes expressed at key stages of oligodendrocyte production. These data indicate that protocadherin 15 (*Pcdh15*) mRNA is more highly expressed by OPC than any other cell type in the CNS, indicating that it may regulate OPC function in some way. PCDH15 is a member of the cadherin superfamily of transmembrane proteins that mediate calcium-dependent cell adhesion. Mutant PCDH15 has been associated with the development of Usher syndrome and has been implicated in the development of certain types of glioma. However, the role of PCDH15 within the normal CNS, and more specifically in the oligodendrocyte lineage is unknown. Using *in situ* hybridization and immunohistochemistry, we have confirmed that *Pcdh15* mRNA and protein are expressed by OPCs and oligodendrocytes. By transfecting primary OPC cultures with lentiviral particles that contain DNA encoding short hairpin RNAs designed to bind *Pcdh15* mRNA, we were able to significantly reduce the level of PCDH15 expressed by these cultures ($p<0.0005$). Furthermore, decreased PCDH15 expression was accompanied by a significant increase in OPC proliferation *in vitro* ($p<0.005$). These data suggest that PCDH15 acts as a negative regulator of OPC proliferation.

Poster 394 – Monday 5th December

TRANSPLANTATION OF EXCITATORY NEURAL PRECURSOR CELLS TO PROMOTE RESPIRATORY RECOVERY AFTER CERVICAL SPINAL CORD INJURY

Lyandysha Zholudeva[1], Nisha Iyer[2], Victoria Spruance[1], Kristiina Hormigo[1], Tatiana Bezdudnaya[1], Itzhak Fischer[1], Shelly Sakiyama-Elbert[2], Michael Lane[1]

[1]Drexel University College Of Medicine, [2]Washington University

The majority of spinal cord injuries occur at the cervical level resulting in persistent, life-threatening respiratory deficits. This can be attributed in large part to the direct compromise of the phrenic motor circuitry that controls the diaphragm – the primary respiratory muscle. Despite this devastating outcome, experimental and clinical studies have demonstrated an intrinsic capacity of the injured spinal cord to exhibit limited spontaneous functional recovery. A key contributor to this plasticity are spinal interneurons, which undergo axonal sprouting, promoting the formation of novel functional neuronal relays. With a primary focus on spinal interneurons, the present study tests whether transplantation of spinal neural precursor cells enriched with excitatory interneurons can provide a cellular substrate that promotes the formation of functional neuronal relays capable of improving phrenic motor recovery.

Neural precursor cells (neuronal and glial restricted progenitor cells) were enriched with stem cell derived Chx10-driven excitatory interneurons and transplanted into a clinically-relevant cervical (C3-4) spinal cord contusion injury in adult rodents. Anatomical connectivity of grafted cells was assessed using a retrograde, transynaptic tracing technique while the functional contribution of grafted cells was analyzed with terminal bilateral diaphragm electromyograms. Anatomical analysis revealed donor cell survival, differentiation and integration with the injured host phrenic circuitry. Functional diaphragm electromyography demonstrated altered patterns of activity one

month following treatment in transplant recipients. These ongoing studies not only test the efficacy of a promising therapeutic strategy, but also offer insight into the neuronal phenotypes that can be effective for neural transplantation into the injured nervous system.

Poster 395 – Tuesday 6th December

PHYSICAL EXERCISE ALTERS THE MICROSTRUCTURE AND CONNECTOME IN THE AGED MURINE BRAIN

Miss Alice(Xiaoqing) Zhou[1], Dr Daniel Blackmore[1], Prof Perry Bartlett[1]

[1]QBI

Objective: Physical exercise is known to cause structural changes in the brain and improve cognitive ability in aged humans. Parallel studies have shown that physical exercise in mice could lead to volume changes in the hippocampus and other parts of the brain, however, little is known about whether exercise alters either the microstructure or the connectome. The aim of this study is to determine whether diffusion tensor imaging (DTI) can robustly monitor these changes following physical exercise in the aged mouse brain.
Methods: 24-month-old C57BL/6 mice underwent voluntary running on a wheel and in vivo diffusion tensor imaging was performed using a 9.4T MRI scanner to compare the changes in microstructure and connectome between exercised animals and sedentary controls.
Key findings: Following exercise, mice displayed significant changes in both microstructure and connectome when compared to controls. Microstructure changes are characterised by a decreased mean diffusivity (MD) value in the hippocampus formation; changes consistent with increased cell density. Connectome changes are characterised by an increase in number and strength of connection, which was most evident in hippocampus and caudate putamen. In a connection-wise comparison, we further revealed strengthened connections between the hippocampus and caudate putamen. These changes were positively correlated with cognitive performance.
Conclusion: This study demonstrates that physical exercise can elicit measurable structural changes in mouse brain using the diffusion MRI modality and these changes may reflect improved functional connectivity in the hippocampus.

Poster 396 – Monday 5th December

CELLULAR CHANGES IN MILD TRAUMATIC BRAIN INJURY BETWEEN YOUNG AND ADULT MICE AFTER LATERAL FLUID PERCUSSION INJURY

Phd Zhendan Zhu[1], Dr Jyoti Chuckowree[1], Dr Catherine Blizzard[1], Assoc Prof Tracey Dickson[1]

[1]Menzies Institute For Medical Research - University Of Tasmania

Mild traumatic brain injury (TBI) has been deemed a 'silent epidemic', with accumulating evidence indicating that such injuries can set in motion an insidious cascade of neural alterations manifesting in ongoing and life-long impairments in neurological and cognitive function. In order to determine the relative vulnerability of the young brain to TBI, we explored both the similarities and differences in the cellular responses following mild TBI in mice across the life span. In this study, cohorts (n=6-8) of young (6 weeks old) and adult (12 weeks old) male Thy1-YFPH transgenic mice were exposed to a mild lateral TBI, using a Fluid Percussion Injury device. Young and adult sham-operated and also Naïve mice (n=6-8) were processed concurrently. Mice were transcardially perfused 7 days following mTBI/sham-operation and the brains were prepared for immunohistochemistry. The expression of GFAP in the ipsilateral brain was examined by quantitating the percentage area occupied by GFAP immunoreactive astrocytes. Results showed that average level of GFAP was not significantly different between the young and adult naïve brains or sham-operated brains. However, there was significantly ($p<0.05$) more area occupied by GFAP immunoreactive astrocytes in the young FPI group (0.60□0.22 %) relative to the adult FPI group (0.27□0.08 %), particularly in the internal capsule. The results from this study indicate distinct differences between the glial response to mild trauma in the young and adult brain. Ongoing investigations will focus on other glial and neuronal populations.

Poster 397 – Tuesday 6th December

NEONATAL OVERFEEDING IMPAIRS GHRELIN SIGNALLING IN FEMALE RATS

Ms Ilvana Ziko[1], Dr Luba Sominsky[1], Ms Thai Xin Nguyen[1], Associate Professor Sarah Spencer[1]

[1]Rmit University

Ghrelin is a major metabolic hormone involved in growth, obesity, and stress. Recent research, including our own, has demonstrated ghrelin's role in coordinating the hypothalamic-pituitary-adrenal (HPA) axis responses to stress, at all levels of the HPA axis. In neonates, ghrelin has an additional role, regulating the development of hypothalamic connectivity that controls feeding. This developmental role of ghrelin within the hypothalamus can be influenced by neonatal diet, at least in males. We have previously shown that overfeeding in early life induces an overweight phenotype that is maintained into adulthood in both male and female rats. We have also seen exacerbated HPA axis responses to psychological stress after neonatal overfeeding in female, but not male rats. In this study we were therefore interested to investigate ghrelin's role in these altered stress responses in neonatally overfed females, by assessing the ability of ghrelin to stimulate the pituitary release of adrenocorticotropic hormone (ACTH) and growth hormone (GH). We also examined whether neonatal overfeeding induces alterations in the hypothalamic ghrelin system similar to those previously seen in males. Our preliminary findings indicate that, unlike in males, neonatal overfeeding in females does not alter the hypothalamic ghrelin system. On the other hand, the pituitary's ability to release ACTH and GH is suppressed in adult females that had been overfed as neonates, potentially due to increased constitutive activity of the receptor for ghrelin, growth hormone secretagogue receptor (GHSR). These data support the growing evidence that significant sex differences exist in the consequences of childhood obesity.

Poster 398 – Monday 5th December

ATTENTIONAL MODULATION OF PREFRONTAL CIRCUITS THAT MEDIATE FEAR EXTINCTION

Roger Marek[1], Jingji Jin[2], Li Xu[1], Stephen Maren[2], & Pankaj Sah[1]

[1]*The University of Queensland, Queensland Brain Institute, Brisbane, Australia*
[2] *Department of Psychology and Institute for Neuroscience, Texas A&M University, College Station, TX, USA*

It is well established that the amygdala, the 'core region' for emotional processes, forms extensive connections with the hippocampus (HPC) and the prefrontal cortex (mPFC) to control extinction learning. In rodents, the prelimbic (PL) and infralimbic (IL) prefrontal cortices both play distinct roles in regulating emotional learning, whereby the hippocampus is crucially involved in regulating the contextual information in which extinction occurs. By using an optogenetic and pharmacogenetic approaches, combined with single cell recordings or behavior, we investigated the neuronal circuit underlying fear extinction both *ex vivo* and *in vivo.*
Ex vivo investigation within the mPFC revealed gutamatergic unidirectional deep-layer projections from the PL to the IL. Moreover, activation of this PL to IL connection during extinction learning was found to enhance extinction learning. This novel finding redefines the role of prefrontal sub-regions in fear extinction.
Hippocampal projections were found to target both pyramidal cells and interneurons in the IL, with a predominant innervation of interneurons. Characterization of these interneurons revealed strongest innervation of fast-spiking, parvalbumin-positive interneurons, which form local feed-forward inhibition of pyramidal cells, some of which project to the amygdala. On the behavioral side, pharmacogenetic modulation of this HPC-IL projection altered context-dependent recall of fear extinction, thereby illustration the importance of this projection in regulating the recall of extinction memory.
Taken together, these findings improve our understanding about the mPFC-driven neural circuitry that underlies the extinction of learned fear.

Poster 399 – Tuesday 6th December

CENTRAL MODULATION OF CARDIAC SYMPATHETIC NERVE ACTIVITY FOLLOWING ACUTE MYOCARDIAL INFARCTION.

Roy R.K., Brown, C.H., Schwenke, D.O.

Department of Physiology, University of Otago, Dunedin, New Zealand.

Acute myocardial infarction (MI) is a global health problem costing around 7.4 million lives every year. One of the main contributing factors to the high mortality associated with acute MI is an adverse increase in cardiac sympathetic nerve activity (SNA). Once established, the sustained increase in cardiac SNA is essentially irreversible, even with the use of sympathetic beta receptor blockers. The increased drive for SNA is most likely central in origin. However, the regions of the brain involved in the generation of increased SNA immediately after acute MI remains unknown. Therefore, we aimed to assess the activation of specific brain regions in response to acute MI. We first determined the specific regions of brain that are activated in the early stages following acute MI. Rats were transcardially perfused under anesthesia 90 min following the induction of MI. Immunohistochemistry for Fos protein was performed on brain sections as a marker of neuronal activation. MI rats had a significantly higher number of Fos-positive cells in the paraventricular nucleus than sham operated rats (P=0.0002, unpaired t-test), which included significantly greater Fos expression in Parvocellular division. We next determined the phenotype of the activated parvocellular neurons using double label immunohistochemistry. Acute MI was associated with significantly higher number of Fos-positive oxytocin (OT) neuron compared to sham (P=0.0022, unpaired t-test). As parvocellular neurons are known to project to rostral ventrolateral medulla (RVLM), next we identified the activated parvocellular OT neuronal projection by injecting a retrograde dye into the RVLM. Significantly higher numbers of retrogradely labeled activated OT neurons were identified in the infarcted rat (P=0.001, unpaired t-test). Taken together these results suggest that the activation of parvocellular pre-autonomic OT neurons may drive increased cardiac SNA in early stages of acute MI.