

# POSTERS



## POS-MON-001

**BONE MORPHOGENIC PROTEIN SIGNALLING ALTERS THE PRECURSOR CELL RESPONSE DURING CUPRIZONE-INDUCED DEMYELINATION**Cate H.S.<sup>1,2</sup>, Sabo J.K.<sup>2</sup>, Merlo D.<sup>2</sup>, Aumann T.D.<sup>2</sup> and Kilpatrick T.J.<sup>1,2</sup><sup>1</sup>Centre for Neuroscience, University of Melbourne. <sup>2</sup>Howard Florey Institute, University of Melbourne.

Oligodendrocyte apoptosis is a key pathological event in CNS demyelination. This leads to demyelination of axons and progressive impairment of nerve cell function. Only limited endogenous remyelination occurs and enhancement of this process by augmenting regeneration of oligodendrocytes is emerging as a promising therapeutic strategy. Our work has revealed that Bone Morphogenic Protein (BMP) signalling is elevated during cuprizone-induced demyelination in two populations of cells that are likely sources of replacement oligodendrocytes, namely subventricular zone (SVZ) neural precursor cells (NPCs) and oligodendrocyte progenitor cells (OPCs). As we have shown that BMP4 modulates the production of astrocytes and oligodendroglia from adult NPCs in culture, we investigated the role of BMP signalling in promoting oligodendroglialogenesis *in vivo*. We used osmotic mini-pumps to infuse BMP4, its endogenous antagonist Noggin or vehicle into the brain during cuprizone-induced demyelination. In cuprizone treated control animals (n=4), GFAP+ astrocytes are increased in the SVZ while proliferating OPCs are increased in the myelin lesion (p<0.05). Noggin infusion was effective in blocking BMP signalling as it reduced levels of phosphorylated SMAD1/5/8, a key component of BMP4 signalling. Noggin infusion also decreased GFAP+ astrocyte numbers compared to vehicle (n=5 p<0.05) providing further *in vivo* evidence that BMP signalling alters astroglial lineage commitment. BMP4 infusion (n=6) increased pSMAD1/5/8 as well as proliferating OPCs in lesions during demyelination (p<0.05). Even so, one-week after recovery, the Noggin infused animals had the highest number of mature oligodendrocytes in lesions. We conclude that, during demyelination, BMP signalling affects the lineage commitment of SVZ NPCs and the proliferation and differentiation of OPCs. Manipulating the timing and activity of the BMP signalling pathway could enhance the numbers of mature oligodendrocytes capable of remyelination.

## POS-MON-003

**AN AGE DIFFERENCE IN MU OPIOID RECEPTOR BINDING DENSITY IN THE HUMAN PUTAMEN BUT NO CHANGES IN SCHIZOPHRENIA**Pan B.<sup>1</sup>, Huang X.-F.<sup>1,2</sup>, Dedova I.<sup>2,3</sup> and Deng C.<sup>1,2</sup><sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia.<sup>2</sup>Schizophrenia Research Institute, Darlinghurst, 2010, NSW, Australia. <sup>3</sup>Discipline of Pathology, University of Sydney, NSW 2006, Australia.

The putamen is strongly implicated in the pathophysiology of schizophrenia as well as its associated therapeutic side effect of tardive dyskinesia. Polymorphisms study has suggested that Mu opioid receptor (MuR) is associated with occurrence of tardive dyskinesia in schizophrenia patients. In this study, we investigated the binding density of MuR in the putamen of schizophrenia patients compared to control subjects. Relationship between MuR binding density and age was also analysed. **METHODS:** Postmortem brain tissue was obtained from the NSW Tissue Resource Centre (TRC) through brain donor program, including 15 schizophrenic patients and 15 matched controls. Quantitative autoradiography was used to investigate the binding of [3H]DAMGO to MuR in the putamen using a Beta-Imager. **RESULTS:** There was no significant difference between schizophrenia and control groups in MuR binding density. MuR binding density was not correlated with postmortem interval, or brain pH, or the final recorded dose of antipsychotic drugs used in schizophrenia patients (p>0.05). However, a positive correlation was observed between MuR binding density and age of subjects (r=0.378, p<0.05). A negative correlation was observed between MuR binding density and age of on-set in schizophrenia patients (r=-0.525, p<0.05). **CONCLUSION:** These results suggest that MuR in the putamen is possibly not involved in the pathophysiology of schizophrenia. However, age is the main factor to influence MuR binding density in the human putamen.

## POS-MON-002

**FRONTAL WHITE MATTER AND POSTERIOR CINGULATE ABNORMALITIES METABOLITE ABNORMALITIES IN OLDER AND CLINICALLY STABLE HIV+ INDIVIDUALS**Cysique L.A.<sup>1</sup>, Moffat K.<sup>2</sup>, Nugent-Cleary-Fox E.<sup>1</sup>, Brew B.J.<sup>1,2</sup> and Rae C.<sup>1,3</sup><sup>1</sup>University of New South Wales, Brain Sciences. <sup>2</sup>St.Vincent's Hospital.<sup>3</sup>Prince of Wales Medical Research Institute.

**Background:** Sub-clinical and Mild forms of HIV-associated neurocognitive disorders (HAND) are more common in the era of combination antiretroviral therapy. **Methods:** 21 HIV+ individuals > 44 years old on treatment with HIV RNA below detection and three age-comparable controls have been enrolled in an ongoing study to investigate aging and HIV effects on the brain. All underwent neuropsychological testing and <sup>1</sup>H magnetic resonance imaging (MRS), including right frontal white matter (RFWM) and posterior cingulate cortex (PCC). MRS quantification was conducted using jMRUI with baseline and water correction. **Results:** All controls and most HIV+ participants performed within the normal neuropsychological range except for three with mild HAND. Because of small sample size, we defined a 90% confidence interval (CI; 2-tailed) around the control's metabolite concentrations. Concentrations for which 30% (1-tailed; effect size=.50) of the HIV+ sample was outside the CI were retained as marker of abnormality. The pattern of abnormalities in HIV+ individuals was: increased FWM Myo-inositol; lower PCC N-Acetyl-Aspartate; increased PCC Myo-inositol; increased PCC Choline/N-Acetyl-Aspartate ratio; increased PCC Myo-inositol. Increased PCC Choline/N-Acetyl-Aspartate ratio correlated with lower overall neuropsychological performance (r=-.63; p=.002) and lower mental flexibility (r=-.57; p=.006); age did not correlate with any abnormal metabolite concentration; depression correlated with increased FWM and PCC Myo-inositol (p<.01). **Conclusion:** These preliminary results showed brain metabolites abnormalities consistent with ongoing neuroinflammation despite HIV viral control and sub-clinical deficits. In this older sample, posterior brain regions seem affected in contrast with the higher brain injury traditionally observed in younger HIV+ individuals.

## POS-MON-004

**IMPACT OF ADULT VITAMIN D<sub>3</sub> (AVD) DEFICIENCY ON BRAIN FUNCTION AND BEHAVIOUR IN SPRAGUE-DAWLEY RATS**Voogt M.<sup>1</sup>, Cui X.<sup>1</sup>, McGrath J.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia. <sup>2</sup>Queensland Centre for Mental Health Research, Wacol, QLD 4067 Australia.

**Purpose:** The incidence of schizophrenia varies across populations and is influenced by genetic and environmental factors. Vitamin D<sub>3</sub> exposure has been proposed as one such environmental risk factor and research in rodents indicates that prenatal vitamin D<sub>3</sub> deficiency affects brain development and behaviour. However, there is little evidence that vitamin D<sub>3</sub> deficiency has an impact on the adult brain. Thus, the focus of this project was to establish, for the first time, an AVD-deficient rat model. **Methods:** Ten-week old male rats were fed a control or vitamin D<sub>3</sub> deficient diet for 6 weeks prior to, and during behavioural testing (n=70). Tissue was collected to analyse brain neurochemistry (n=16). A separate group of rats were tested for their response to the psychomimetics, D-amphetamine and MK-801 (n=42). **Results:** After 8-10 weeks on the diet, AVD-deficient rats were deficient in vitamin D<sub>3</sub> and had normal calcium and phosphate levels. AVD deficiency was associated with a subtle behavioural phenotype, including enhanced PPI and a transient decrease in sensitivity to the locomotor effects of D-amphetamine. Specific changes in brain neurochemistry included altered serotonin and noradrenaline content in the amygdaloid complex and brainstem. Finally, decreased dopamine and serotonin turnover was observed in the prefrontal cortex. **Conclusions:** Although these data indicated a subtle phenotype for AVD-deficient rats, this model did not demonstrate many features typically associated with classical animal models of schizophrenia. The changes observed using this protocol suggest that further refinement to the model is necessary to evaluate the role of vitamin D<sub>3</sub> deficiency on the adult brain.

## POS-MON-005

**MODELLING COGNITIVE SYMPTOMS OF SCHIZOPHRENIA IN DEVELOPMENTAL VITAMIN D (DVD)-DEFICIENT RATS**Turner K.M.<sup>1</sup>, McGrath J.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD.<sup>2</sup>Queensland Centre for Mental Health Research, Wacol, QLD.

**Purpose:** Epidemiological evidence suggests that vitamin D may be a potential risk factor for several neuropsychiatric disorders, including schizophrenia, and we have shown that it is biologically plausible using a developmental vitamin D (DVD)-deficient rat model. While hallucinations and delusions (positive symptoms) feature prominently in diagnostic criteria, impairments of attentional processing (cognitive symptoms) are a cardinal feature of schizophrenia. Our aim was to investigate cognitive processing and neurobiological alterations in DVD-deficient rats. **Methods:** Six-month old DVD-deficient and control rats were assessed on selected cognitive domains using a 5 choice continuous performance task in an operant chamber. Brief flashes of light signalled the rat to either respond or withhold responding to receive a food reward. At the end of the experiment dopamine and metabolites were measured in brain tissue using HPLC. **Results:** Performance was not altered in response trials. On withhold trials, control rats (n=12) were able to inhibit responding in 40% of trials, whereas DVD-deficient rats (n=16) only suppressed their response on 10% of trials (p<0.05). This finding persisted over repeated testing sessions (14 days). No changes were detected in the prefrontal cortex but there were significant reductions in dopamine and metabolites in the hippocampus (p<0.05). **Conclusions:** While DVD-deficient rats were normal on all measures of vigilance (on response trials), their lack of inhibition on withhold trials was observed immediately and persisted throughout testing. The DVD-deficient rat model is characterised by a phenotype reminiscent of both positive and negative symptoms of schizophrenia, and these experiments suggest that DVD-deficient rats have cognitive impairments as well.

## POS-MON-006

**DOES VITAMIN D DEFICIENCY ALTER ATTENTIONAL PROCESSING IN RATS?**Byrne J.H.<sup>1</sup>, Turner K.<sup>1</sup>, Voogt M.<sup>1</sup>, McGrath J.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD4072 Australia. <sup>2</sup>Queensland Centre for Mental Health Research, Wacol, QLD 4076 Australia.

**Purpose:** Epidemiological evidence suggests that periods of vitamin D deficiency during gestation or adulthood may contribute to impairments in cognition. We have developed two rodent models to test the biological plausibility that low levels of vitamin D impacts on aspects of attentional processing, including the developmental vitamin D (DVD) and adult vitamin D (AVD) deficiency models. The aim of this study was to investigate attentional processing and working memory in both DVD- and AVD-deficient rats, and then probe neurotransmitter systems involved in attentional processing. **Methods:** Sprague-Dawley rats were either fed a vitamin D deficient diet during gestation (DVD) or as adults (AVD) for a minimum of 6 weeks and compared with control rats that were fed a diet containing vitamin D. The rats were assessed as adults on selected cognitive domains (attention and speed of processing, learning and memory, and problem solving) using the 5 choice serial reaction time task, in which the rat was required to correctly respond to brief flashes of light to receive a food reward. At the end of the experiment brains were removed and regional levels of catecholamines were assessed in prefrontal cortex, hippocampus and striatum using HPLC. **Results:** DVD-deficiency resulted in a subtle behavioural phenotype on measures of attentional processing in terms of accuracy and speed of performance. DVD-deficient rats were significantly more impulsive than control rats, and preliminary data indicates that levels of dopamine and metabolites are reduced in the hippocampus of DVD-deficient rats, whereas dopamine turnover was reduced in the prefrontal cortex of AVD-deficient rats, but not vice versa. **Conclusions:** Taken together it appears that vitamin D deficiency impacts on specific aspects of attentional processing (impulsivity). Although these changes seem to be correlated with altered dopamine signaling the specific changes were dependent on the period of vitamin D deficiency (during development or adulthood), and support the notion that low levels of vitamin D may have adverse effects on cognitive performance in rodents.

## POS-MON-007

**DEVELOPMENTAL VITAMIN D (DVD) DEFICIENCY IS ASSOCIATED WITH BEHAVIOURAL ALTERATIONS THAT ARE RELEVANT TO SCHIZOPHRENIA**Burne T.H.J.<sup>1,2</sup>, Turner K.<sup>1</sup>, Alexander S.<sup>2</sup>, Kesby J.P.<sup>3</sup>, McGrath J.J.<sup>1</sup><sup>2</sup> and Eyles D.W.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute. <sup>2</sup>Queensland Centre for Mental Health Research. <sup>3</sup>School of Biomedical Sciences, University of Queensland.

**Purpose:** It is now recognized that vitamin D is active in the brain and plays an important role in brain development. Guided by certain features of the epidemiology of schizophrenia, we have explored the role of vitamin D in the developing brain and behaviour using a rodent model of developmental vitamin D deficiency (DVD). Our aim was to investigate the behaviour of mature adult rats on tests of locomotion, sensorimotor gating, social interaction and attentional processing, under baseline conditions and in response to the NMDA receptor glutamate antagonist, MK-801. **Methods:** Sprague-Dawley rats were fed a vitamin D deficient diet or control diet 6 weeks prior to mating until birth when they were maintained on a diet containing vitamin D until adulthood. The behavioural phenotype of separate groups of 6-month old offspring (n=8-12 per group) was assessed in an open field, prepulse inhibition of the acoustic startle response, social interaction or using the 5 choice serial reaction time task, as a measure of vigilance, under baseline conditions and in response to saline or different doses of MK-801 (0.05-0.5 mg/kg). **Results:** The behavioural phenotype of DVD rats included specific alterations in response to MK-801 on several aspects of tests of locomotion, sensorimotor gating, social interaction and attentional processing (Main effect of diet p<0.05). There was a significant interaction between prenatal diet and sex on tests of sensorimotor gating and social interaction, but not on tests of locomotion or attentional processing. **Conclusions:** In summary, low prenatal levels of vitamin D can influence critical components of orderly brain development. The behavioural phenotype of DVD-deficient rats is subtle, but incorporates features that are relevant to the positive, negative and cognitive symptoms of schizophrenia.

## POS-MON-008

**SENSORIMOTOR GATING IN MATURE ADULT DEVELOPMENTALLY VITAMIN D (DVD)-DEFICIENT RATS**Alexander S.A.<sup>2</sup>, McGrath J.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD4072. <sup>2</sup>Queensland Centre of Mental Health Research, Wacol, QLD 4076.

**Purpose:** Epidemiological evidence suggests that vitamin D deficiency during gestation may be a risk factor for schizophrenia. Using a rodent model of developmental vitamin D (DVD) deficiency we have shown long lasting changes in terms of brain development and behaviours, including hyperlocomotion and locomotor sensitivity to the NMDA antagonist, MK-801. The aim of this study was to examine prepulse inhibition (PPI) of the acoustic startle response (ASR) in the mature adult (6 month old) DVD-deficient rat and to verify whether PPI responses were impaired by MK 801. **Methods:** Sprague-Dawley rats were fed a vitamin D deficient diet 6 weeks prior to mating until birth when they were transferred to a vitamin D containing diet until testing. Control rats were fed a diet containing vitamin D throughout the experiment. The rats were group housed (2-4) and tested for ASR and PPI at 6 months of age under baseline conditions or after treatment with saline, low dose or high dose MK-801. **Results:** Baseline ASR and PPI responses were not affected by prenatal diet. Low dose MK-801 resulted in a significant increase in ASR in female DVD-deficient rats but did not selectively affect PPI. MK-801 had no effect on ASR in male DVD-deficient rats however high doses of MK-801 selectively impaired PPI. **Conclusions:** These data reveal a complex interaction between prenatal diet, sex and dose. The results suggest that low prenatal vitamin D can result in long term changes of the neurotransmitter systems governing sensorimotor gating that are affected by psychomimetic drugs, such as MK-801.

## POS-MON-009

**NEUREGULIN1 AND ERBB4 PROTEIN EXPRESSION IN THE RAT BRAIN FOLLOWING PERINATAL PHENCYCLIDINE TREATMENT**Warren C.R.<sup>1,2</sup>, Newell K.A.<sup>1,2</sup>, Du Bois T.M.<sup>1,2</sup> and Huang X.F.<sup>1,2</sup><sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia.<sup>2</sup>Schizophrenia Research Institute, 384 Victoria St, Darlinghurst, 2100, NSW, Australia.

Schizophrenia is a severe psychiatric disorder of unknown aetiology. NMDA receptor hypofunction is a key theory on the cause of schizophrenia. Similarly, Neuregulin1 (NRG1) and its receptor, ErbB4, are strongly implicated in schizophrenia pathology. It is unclear how these systems interact in schizophrenia or in animal models of the disorder. Design: Male and female rat pups (n=5/group) were treated with the NMDA receptor antagonist PCP (10 mg/kg) or saline on postnatal days (PN) 7, 9 and 11. Rats were euthanized on PN12 (juvenile), 35 (adolescent) and 140 (adult) and brain tissue immediately collected for analysis. NRG1 and ErbB4 protein levels were determined by Western blotting. Results: Higher levels of NRG1 and ErbB4 protein expression were observed at early developmental time-points compared to adults. Compared to male control rats, female controls expressed higher levels of ErbB4 protein at PN12 in the anterior cingulate cortex and hippocampus (p's<0.01) and at PN140 in the anterior cingulate cortex and prefrontal cortex (p's<0.01). Perinatal PCP treatment reduced NRG1 (p<0.01) but increased ErbB4 protein expression (p<0.05) in the PFC of adult males but not females. Conclusions: Perinatal PCP treatment can induce long-term age, gender and brain region-specific alterations in the expression of NRG1 and ErbB4 protein in the rat brain. This may suggest that altered NRG1/ErbB4 levels in the schizophrenic brain could be a secondary effect of NMDA receptor hypofunction via PSD-95 for example. Further research should investigate these important findings and their implications for the aetiology of schizophrenia.

## POS-MON-011

**ENVIRONMENTAL ENRICHMENT AFFECTS PCP-INDUCED BEHAVIOURAL AND NEUROCHEMICAL SYMPTOMS OF SCHIZOPHRENIA**Frank E.<sup>1,2</sup>, Snikeris P.<sup>1,2</sup>, Pathy R.<sup>1,2</sup> and Huang X.F.<sup>1,2</sup><sup>1</sup>Schizophrenia Research Institute, Sydney, NSW, Australia. <sup>2</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, NSW, Australia.

Schizophrenia is increasingly accepted as neurodevelopmental disorder, which is getting shaped by the rearing environment of the individual. Here, we investigated the effects of different rearing conditions (enrichment vs deprivation) on the development of behavioural and neurochemical schizophrenia-like characteristics in the perinatal PCP mouse model for schizophrenia. C57Bl/6 mice were treated on post-natal days (PND) 5, 7, 9 and 11 with either PCP (10mg/kg) or saline and then assigned at PND 21 to either (1) isolation, (2) enriched or (3) standard housing. At 8 weeks of age, animals were tested for anxiety-related and depression-like behaviours (n=12), their neuroendocrine stress reactivity (n=6) as well as NMDA and GABAA receptor density in the prefrontal cortex, striatum and hippocampus (n=6). Under standard housing conditions, PCP-treated male mice showed increased depression-like behaviour, which was comparable to isolation reared animals, independent of their treatment. Intriguingly, rearing in enriched environment reduced the depression-like behaviour of PCP-treated males to a level comparable to saline treated animals housed under standard conditions. Anxiety-related behaviour and neuroendocrine parameters remained unchanged. NMDA receptor density was partially up-regulated due to PCP-treatment, whereas GABAA receptors were found down-regulated due to enrichment. According to the concept of experience-dependent plasticity, rearing in an enriched environment was shown to compensate the PCP-induced depression-like behaviour; in contrast, isolation housing was shown to be as detrimental as perinatal PCP-treatment. Ongoing studies will explore changes in cognition as well as neuroimmunological factors.

## POS-MON-010

**STUDYING THE 'TWO-HIT' HYPOTHESIS OF SCHIZOPHRENIA IN MICE: ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)**

Klug M., Choy K.H.C., Hill R. and Van den Buuse M.

Mental Health Research Institute, 155 Oak Street, Parkville, VIC 3052, Australia.

Epidemiological studies have suggested that schizophrenia is caused by an early disruption, such as a genetic deficit or environmental stress, which increases vulnerability to late factors, such as drug abuse or social stress, i.e. the 'two-hit' hypothesis. We aimed to study the central mechanisms involved in the interactive effects of early and late neurodevelopmental factors on cognition in adulthood. Previous work showed that rats that had undergone early stress combined with chronic young-adult corticosterone (CORT) treatment, exhibited disrupted short-term spatial memory and a significant ~25% reduction of BDNF expression in the dentate gyrus, and approximately 20% reductions in the CA1 and CA3 (Choy et al., Hippocampus, 2008). To further assess the relationship between reduced BDNF levels and neurodevelopmental stress, we used male and female BDNF heterozygous mutant mice (Het), which show marked reduction of BDNF levels in the brain. As a second stressor mice received CORT in their drinking water from 6-9 weeks of age. The mice (n=11-18 in each group) were behaviourally tested at 11 weeks of age. Control groups, i.e. wild-type mice, wild-type mice receiving CORT, or BDNF Het mice, showed intact Y-maze spatial memory, as evidenced by a significant preference to spend time in the novel arm one- or two hours after the 2-arm pre-exposure. In contrast, male CORT-treated BDNF Het mice showed no significant preference for the novel arm, suggesting disrupted short-term spatial memory. The disruption was not seen in female 'two-hit' mice. These results may help to explain the development of cognitive deficits in patients with mental illnesses with a neurodevelopmental origin, such as schizophrenia.

## POS-MON-012

**CANNABINOID CB1 RECEPTOR DENSITY IS INCREASED IN THE DORSOLATERAL PREFRONTAL CORTEX (BRODMANN'S AREA 46) IN SCHIZOPHRENIA**Dalton V.S.<sup>1,2</sup> and Zavitsanou K.<sup>2,1</sup><sup>1</sup>Schizophrenia Research Institute, Sydney, Australia. <sup>2</sup>Australian Nuclear Science and Technology Organisation, Sydney, Australia.

Previous studies have indicated that cannabis use is associated with an increased risk of developing schizophrenia and can exacerbate psychotic symptoms in schizophrenic patients. Furthermore, results from experiments with post-mortem human brain tissue suggest that expression of the cannabinoid CB1 receptor is increased in regions such as the anterior and posterior cingulate cortices and Brodmann's area 9 in schizophrenia. We examined CB1 receptor density in the dorsolateral prefrontal cortex (Brodmann's area 46), a region associated with working memory deficits in schizophrenic patients. Receptor density was investigated in this area using in vitro autoradiography with the CB1 receptor ligand [<sup>3</sup>H] CP55,940 in a large cohort of schizophrenic (n=30), schizoaffective (n=7) and control (n=37) cases matched for age, gender, pH and postmortem interval. Results were analysed using ANCOVA controlling for age, pH, freezer storage time and brain volume. A 10% increase of borderline significance in CB1 receptor density was found when binding in the schizophrenic and schizoaffective group was compared to controls (p=0.058, F=3.706, df=1). This increase reached significance (p=0.024; F=5.33; df=1) when schizoaffective cases were removed from the analysis. Factors such as post-mortem interval time, gender and agonal state were not found to have an effect on CB1 binding. Within the schizophrenic and schizoaffective group, CB1 binding was not affected by antidepressant history and the final recorded antipsychotic drug dose. No difference in CB1 receptor binding was found in patients that had committed suicide compared to those that died of natural causes. These results suggest that alterations in the endogenous cannabinoid system in Brodmann's area 46 may be involved in the pathology of schizophrenia particularly with regard to working memory deficits and other negative symptoms.



## POS-MON-013

# THE EFFECTS OF THE SYNTHETIC CANNABINOID HU210 ON [35S]TBPS BINDING TO GABA(A) RECEPTORS IN THE BRAIN OF ADULT AND ADOLESCENT RATS

Verdurand M.<sup>1,2</sup>, Dalton V.S.<sup>1,2</sup> and Zavitsanou K.<sup>1,2</sup>

<sup>1</sup>Schizophrenia Research Institute, Sydney, Australia. <sup>2</sup>Australian Nuclear Science and Technology Organisation, Sydney, Australia.

Cannabinoids are known to induce transient psychotic symptoms and cognitive dysfunction in healthy individuals and contribute to trigger schizophrenia in vulnerable individuals, particularly during adolescence. Converging preclinical evidence suggests important interactions between cannabinoid and GABAergic systems. **Aim:** In the present study we compared the effects of cannabinoid treatment on GABA(A) receptor binding in the brain of adolescent and adult rats. **Methods:** Adolescent (5 weeks old) and adult (10 weeks old) rats were treated with the synthetic cannabinoid HU210 (25, 50 or 100 µg/kg/day) or vehicle for 1, 4 or 14 days. Rats were sacrificed 24 hours after the last injection and GABA(A) receptor density was measured in several brain regions using [<sup>35</sup>S]TBPS and *in vitro* autoradiography. **Results:** In the adult rats, 14 days treatment with 50 and 100 µg HU210 significantly increased GABA(A) receptors in dentate gyrus by 20% (P=0.015) and by 22% (P=0.006), respectively, whereas 14 days treatment with 100 µg increased GABA(A) receptors by 19% in CA1 region (P=0.038). HU210 did not affect GABA(A) receptors in adolescent rats in any treatment regimen and in adult rats treated with HU210 for 1 or 4 days. **Conclusion:** These data suggest that long-term high-dose treatment with HU210 increases GABA(A) receptors in the hippocampus of adult rats, possibly as compensation to reduced GABA release reported in the same brain region by others after exposure to cannabinoids. Such changes may interfere with associated cognitive functions. In addition, our results suggest that the adolescent brain does not display the same compensatory mechanisms that are activated in the adult brain following cannabinoid treatment.

## POS-MON-014

# THE EFFECTS OF THE SYNTHETIC CANNABINOID HU210 ON 5-HT1A RECEPTOR MRNA EXPRESSION IN THE BRAIN OF ADULT AND ADOLESCENT RATS

Wang H.Q., Nguyen V. and Zavitsanou K.

Radiopharmaceuticals Research Institute, Australian Nuclear Science and Technology Organisation, PMB 1 Menai, Sydney, NSW, 2234, Australia.

Cannabinoids are known to interact with brain systems implicated in psychosis, such as the serotonin (5HT) system and also to trigger psychosis in vulnerable individuals, particularly during adolescence. **Aim:** The aim of this study was to compare the effects of cannabinoids treatment on 5-HT1A receptor mRNA expression in the brain adolescent and adult rats. **Methods:** Adolescent (5 weeks old) and adult (10 weeks old) rats were treated with the synthetic cannabinoid HU210 (25, 50 or 100 µg/kg) or vehicle for 1, 4 or 14 days. Rats were sacrificed 24 hours after the last injection and serotonin receptor 5-HT1A mRNA expression was measured in several brain regions using *in situ* hybridization. **Results:** Adolescent animals had higher levels of 5-HT1A receptor mRNA expression in CA1 region (38%, P=0.001) and dentate gyrus (37%, P<0.001) of the hippocampus compared to the adults. In the adult rats 4 days treatment with HU210 (100ug/kg/day) significantly increased 5-HT1A receptor mRNA expression in CA1 region (27%, P=0.001) and dentate gyrus (14%, p=0.036) of the hippocampus. No significant differences were observed between adult rats treated with HU210 for 1 or 14 days and vehicle treated controls. HU210 did not affect 5-HT1A receptor mRNA expression in the brain of adolescent rats in any of the treatment regimens examined. **Conclusion:** These data suggest that adolescent rats do not display the same compensatory mechanisms that are activated in the adult brain following cannabinoid treatment and that cannabinoids have the potential to influence hippocampal serotonergic function.

## POS-MON-015

# THE EFFECT OF PERINATAL AND ADOLESCENT BRAIN DEVELOPMENT DISRUPTION FROM NMDA RECEPTOR ANTAGONISM ON CB1 RECEPTOR LEVELS IN THE RAT PREFRONTAL CORTEX

Dawson A.E.<sup>1,2</sup>, Newell K.A.<sup>1,2</sup>, Ruthirakumar S.<sup>1,2</sup> and Huang X.F.<sup>1,2</sup>

<sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia.

<sup>2</sup>Schizophrenia Research Institute, Darlinghurst, 2010, NSW, Australia.

The NMDA receptor (NMDA-R) plays a key role in both perinatal and adolescent brain development. Adverse impacts to the NMDA-R produce long-term neurochemical alterations in rats, mimicking characteristic schizophrenia neuropathology. The effect of NMDA-R antagonism on the cannabinoid receptor (CB1-R) system is largely unknown. **Methods:** Experiment 1: Brains from perinatal MK-801-treated (0.5mg/kg, day 7, 9, 11) and saline (control) female rats were collected at the juvenile, adolescent and adult time points (n=6/group). Experiment 2: Female rats treated with MK-801 at the perinatal (0.5mg/kg, day 7, 9, 11), adolescent (0.3mg/kg, day 42, 44, 46) or both perinatal and adolescent time points, or saline (control) were sacrificed at adulthood (n=5/group). Receptor autoradiography was used to measure CB1-R levels in the prefrontal cortex. **Results:** Experiment 1: CB1-R levels increased from the juvenile to adolescent and adult time points during development however there was no treatment effect of perinatal NMDA-R antagonism at any age. Experiment 2: An increase in CB1-R levels was observed in the double MK-801-treated group compared to the control group and perinatal treatment group. No other groups were significantly different to the control group. **Conclusion:** CB1-R levels are affected by a double but not single NMDA-R antagonist hit in the female rat brain. The result from this double-hit animal model is analogous to the increase in CB1-R levels reported in the prefrontal cortex of human schizophrenia post-mortem tissue and further demonstrates a possible role for the cannabinoid system in the pathogenesis of schizophrenia.

## POS-MON-016

# MOLECULAR PATHWAYS ASSOCIATED WITH PSYCHOSTIMULANT ADDICTION

Brown A.L.<sup>1,2</sup>, Flynn J.R.<sup>1,2</sup>, Smith D.W.<sup>1,2</sup> and Dayas C.V.<sup>1,2</sup>

<sup>1</sup>School of Biomedical Sciences & Pharmacy, Centre for Brain & Mental Health Research, University of Newcastle. <sup>2</sup>HMRI, NSW, Australia.

**Purpose:** Despite intensive research efforts, effective pharmacotherapies for the treatment of psychostimulant addiction do not exist. One possibility for this lack of an efficacious pharmacotherapy is that we still do not fully understand the molecular substrates of addiction susceptibility. Importantly, animal models chosen for study must be clinically valid and, therefore, recapitulate all stages of the addiction process, particularly the important relapse phase. Using an animal model that more accurately reflects drug addiction in humans, we have characterized molecular pathways associated with addiction vulnerability. **Methods:** Sprague Dawley rats (n=60) were trained to self-administer cocaine. Animals were then behaviourally phenotyped into either addiction vulnerable (n=6) or resilient (n=6) groups using adapted DSMIV criteria for addiction (Deroche-Gamonet et al 2004). Gene expression profiles were characterized and molecular pathways elucidated using Gene Set Enrichment Analysis in dorsal (DS) and ventral striatum (VS). **Results:** Preliminary analysis revealed 17 out of 120 gene sets were enriched (FDR < 15%) in VS of 'addicted' rats. In contrast, there were no enriched gene sets in DS of 'addicted' rats. Significantly enriched gene sets potentially relevant to cellular processes underpinning addiction included the mTOR signaling pathway, long-term depression, gap junction, focal adhesion and ERBB signaling pathways. **Conclusions:** Using an animal model of addiction with clinical validity, we found significant enrichment of gene sets within the VS, particularly concerning pathways involved with synaptic plasticity, cell communication and signal transduction. One candidate of interest is mTOR, which has been shown to regulate the synthesis of proteins at active synapses. These studies have elucidated potential pathways that could be of therapeutic value.

## POS-MON-017

## GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IS UNALTERED IN THE ANTERIOR CINGULATE FROM SUBJECTS WITH MOOD DISORDERS

Brooks L.<sup>1,2</sup>, Gibbons A.S.<sup>1</sup> and Dean B.<sup>1</sup><sup>1</sup>The Rebecca L. Cooper Laboratories, The Mental Health Research Institute, Parkville, Victoria, Australia. <sup>2</sup>The School of Biomedical Sciences, The University of Nottingham, Nottingham, UK.

**Background:** We reported increased transmembrane TNF (tmTNF), but not the cleaved soluble TNF (sTNF), in the dorsolateral prefrontal cortex (DLPFC) from subjects with major depressive disorders (MDD)<sup>1</sup> and have shown increased tmTNF in the anterior cingulate cortex (ACC), but not DLPFC, from subjects with bipolar disorder (BPD: data not shown). As TNF is predominantly expressed by neuroglia we have now measured the astrocytic marker, GFAP, to determine if there are generalised changes in astrocytic protein expression in MDD and BPD. **Methods:** Western blots were used to measure levels of GFAP in ACC from 10 subjects with BPD, 10 subjects with MDD and 10 age sex matched control subjects. **Results:** There were no significant changes in the intensities of any of the four GFAP immunogenic bands of molecular weights 37kDa (mean  $\pm$  SEM: BPD =  $1.01 \pm 0.52$  vs. MDD =  $1.17 \pm 0.31$  vs. Controls =  $1.05 \pm 0.31$  ratio internal control;  $p = 0.62$ ), 41kDa (BPD =  $0.74 \pm 0.34$  vs. MDD =  $0.99 \pm 0.33$  vs. Controls =  $1.06 \pm 0.51$ ;  $p = 0.24$ ), 47kDa (BPD =  $0.74 \pm 0.60$  vs. MDD =  $1.33 \pm 0.87$  vs. Controls =  $1.36 \pm 0.81$ ;  $p = 0.20$ ) and 50kDa (BPD =  $2.02 \pm 2.28$  vs. MDD =  $2.71 \pm 1.96$  vs. Controls =  $2.73 \pm 2.14$ ;  $p = 0.70$ ) with diagnoses. **Conclusions:** Our data shows that levels of GFAP do not differ in ACC from subjects with mood disorders and suggest changes in tmTNF in that region in BPD are not associated with generalised changes in levels of astrocyte proteins. <sup>1</sup>Dean B et al (In Press) J.Affect.Dis. 10.1016/j.jad.2009.04.027 [doi].

## POS-MON-019

## SUBJECTIVE MEASURES OF TREATMENT OUTCOME FOR PEOPLE WITH SCHIZOPHRENIA ON ANTIPSYCHOTIC MEDICATIONS

Bakas T.<sup>1,2</sup> and Hinton T.<sup>1,2</sup><sup>1</sup>University of Sydney, NSW 2006. <sup>2</sup>Schizophrenia Research Institute, NSW 2010.

**Purpose:** To investigate variability in outcomes and treatment response to antipsychotics as mediated by the perceived pharmacological action by the individual diagnosed with schizophrenia. **Methods:** A questionnaire consisting of subjective scales was sent to outpatients diagnosed with schizophrenia. The survey pack assessed the variables: symptom severity, medication side-effects, attitudes to treatment, quality of life (QoL), psychosocial function, neuro-cognitive deficits, coping skills, parental bonding and personality. Objective clinical measures of symptom severity, neuro-cognitive deficits and functioning were also examined and contrasted. A reliability test was used to assess internal consistency. Multivariate analysis of variance (MANOVA) was performed, with factors including antipsychotic-induced dysphoria and drug compliance, and dependent variables of symptoms, side effects, functioning and QoL. Multiple linear regression (MLR) was used to assess QoL and the contribution of symptoms, side effects, psychosocial functioning and treatment attitudes upon the QoL measures. **Results:** Reliability was upheld across the scales and subscales assessed within this sample population ( $n=242$ ), with Cronbach's alpha ranging from 0.6-0.9. MLR revealed 69% of variance in QoL was accounted by symptoms, side effects, treatment attitudes and by psychosocial functioning ( $p<0.01$ ,  $n=242$ ). Participants were further divided on the basis of subjective negativity towards treatment (ie: dysphoric vs non-dysphoric responses), where the subjectively negative participant appeared to have more severe symptoms, side-effects and a significantly reduced QoL ( $p<0.01$ ), as did the non compliant participant. **Conclusions:** Subjective evaluation of antipsychotic action leads to differential treatment outcomes for symptoms, side effects and QoL. These results show that self-report measures can be quantified reliably and may provide unique insight into patients with schizophrenia. Such measures may be used to evaluate treatments, both pharmacological and non-pharmacological. This will allow for an assessment of broader outcomes than just symptomatic improvement, such as treatment attitudes and compliance, psychosocial functioning and quality of life.

## POS-MON-018

## ASTROCYTIC TUMOUR NECROSIS FACTOR UNDERLIES NEURON FUNCTION IN COGNITION

Anscomb H.L. and Baune B.T.

School of Medicine &amp; Dentistry, James Cook University, Townsville, Queensland, 4811, Australia.

Pro-inflammatory cytokines have been demonstrated to have a diverse range of actions on the functioning of the CNS, and in particular learning and memory behaviours. Details of the mechanisms of action of cytokines are still to be determined. **Purpose:** This study uses immunohistochemistry techniques (IHC) to investigate cellular changes present in the hippocampal formation as a result of up-regulation of astrocyte-produced tumour necrosis factor (TNF) $\alpha$  (GFAP-TNF $\alpha^{+/+}$ ), prior to onset of behavioural deficits. These findings are compared directly to the hippocampal formation of a TNF $\alpha$  knock-out model (TNF $\alpha^{-/-}$ ) in which marked alterations in learning and memory are observed at the same time-point (12 wks) and to age-match wild-type mice (WT). This time period is of critical importance for further elucidating the role of TNF $\alpha$  in hippocampal dependent learning and memory. **Methods:** Hippocampi from TNF $\alpha^{-/-}$ , GFAP-TNF $\alpha^{+/+}$  and WT ( $n = 5$ ) were subjected to indirect IHC for the analysis of TNF $\alpha$  levels and distribution in regions CA1, CA3 and the dentate gyrus (DG). **Results:** In GFAP-TNF $\alpha^{+/+}$  there was a demonstrated accumulation of TNF $\alpha$  in hippocampal neurons prior to the onset of hippocampal-dependent behavioural deficits. GFAP-TNF $\alpha^{+/+}$  mice also showed a significant increase in TNF $\alpha$  in regions CA3 and the DG ( $p = <0.05$ ) when compared to WT and TNF $\alpha^{-/-}$  mice. WT mice demonstrated immunoreactivity of TNF $\alpha$  in regions CA1 and the DG. **Conclusion:** These findings suggest that astrocyte-produced TNF $\alpha$  is essential for normal development and functioning of the CA1 region of the hippocampus in cognitive processes. However, an overproduction of astrocytic TNF $\alpha$  accumulates in the neurons of the CA3 and DG regions and likely produces functional deficits, as seen in 6 months plus mice, through these regions.

## POS-MON-020

## SECRETASE EXPRESSION AND NEUREGULIN 1 PROCESSING IN SCHIZOPHRENIA

Barakat A.<sup>1</sup>, Scarr E.<sup>2</sup>, Dean B.<sup>2</sup> and Evin G.<sup>1,2</sup><sup>1</sup>Department of Pathology, University of Melbourne, Parkville 3010.<sup>2</sup>Mental Health Research Institute, Parkville 3052.

**Background and Hypothesis:** Schizophrenia (SCZ) is a complex neurological illness that affects 1% of the population. The molecular bases contributing to its pathology remain poorly understood. Genetic studies have linked NRG1 polymorphism to SCZ. Recent studies with mouse models have demonstrated that impaired NRG1-erbB signalling, due to knockout of BACE1 or of the gamma-secretase subunit, Aph1B gene leads to SCZ-like phenotypes that can be rescued by antipsychotics. We hypothesized that the expression of BACE1 and Aph1B, and the proteolytic processing of NRG1 may be altered in the prefrontal cortex of patients with SCZ. **Methods:** Samples from Brodmann 6 region (20 SZ with normal levels of M1 muscarinic receptor; 20 SZ with low levels of M1 muscarinic receptor; 20 age-matched healthy controls - HC) were homogenized with TRLzol and the protein analysed by western blotting for BACE1, Aph1B and NRG-1. Band density was quantified relative to actin. Data were analysed with SPSS software using ANOVA and a significance  $p$  value of  $<0.05$ . **Results:** Protein levels of BACE1, Aph1B, and NRG1 full-length did not differ significantly between the three groups. In contrast, ~ 50 % decrease in NRG-1 CTF was observed in both SCZ groups compared to the HC group ( $p < 0.001$ ). There was a positive correlation between BACE1 and NRG-1 CTF in the HC group, but not in the SCZ groups. **Conclusions:** Our data suggest that the proteolytic processing of NRG-1 is impaired in SCZ. The molecular mechanisms that underlie the decrease in NRG-1 CTF remain to be elucidated.

## POS-MON-021

# INVESTIGATION OF THE NEUROANATOMICAL SUBSTRATES UNDERLYING PRIMED REINSTATEMENT OF A COCAINE-INDUCED PLACE PREFERENCE

Brown R.M.<sup>1,2</sup>, Short J.L.<sup>2</sup> and Lawrence A.J.<sup>1,3</sup>

<sup>1</sup>Howard Florey Institute, University of Melbourne, Parkville, Vic, 3010.

<sup>2</sup>Monash Institute of Pharmaceutical Sciences, Parkville, Vic, 3052.

<sup>3</sup>Centre for Neuroscience, University of Melbourne, Parkville, Vic, 3010.

Vulnerability to relapse is a hallmark characteristic of addiction. Relapse can be modelled in animals using reinstatement models of drug-seeking. This study examined expression of Fos, a marker of neuronal activation, in the brains of mice which exhibited reinstatement of conditioned place preference (CPP) following a cocaine prime (R mice), compared with those which received the drug prime but did not reinstate (NR mice). Adult male mice on a CD1 background were alternately injected with either cocaine (20mg/kg/i.p) or saline and confined to their respective (cocaine or saline paired) compartment in order to induce a CPP. Mice were subsequently extinguished by pairing saline injection with the previously cocaine-paired compartment. Once extinguished, CPP was reinstated by administration of a cocaine prime (10mg/kg/i.p.). R mice (n=16) showed clear reinstatement of a preference for the previously cocaine-paired compartment whereas NR mice (n=11) were not different to extinction. Brains of R (n=8) and NR (n=7) mice were assessed for expression of Fos following the reinstatement session. Activation of the infralimbic cortex, bed nucleus of the stria terminalis, paraventricular nucleus of the hypothalamus, lateroanterior nucleus of the hypothalamus and lateral habenula was significantly correlated with reinstatement propensity ( $p < 0.05$ ), implicating these regions in this behaviour. In addition, no correlation was observed between reinstatement of CPP and either the strength of the original CPP, the development of sensitization to cocaine during conditioning, the time course of extinction, or the expression of psychomotor sensitization following drug prime, suggesting that these behaviours are dissociable from the propensity to exhibit drug-seeking under this paradigm.

## POS-MON-022

# MOLECULAR PROFILE OF STRESS-RELATED REGIONS OF RAT BRAIN FOLLOWING INESCAPABLE FOOT SHOCK

Barreto R.A.<sup>1,2</sup>, Walker F.R.<sup>1,2</sup>, Dunkley P.R.<sup>1,2</sup>, Day T.A.<sup>1,2</sup> and Smith D.W.<sup>1,2</sup>

<sup>1</sup>School of Biomedical Sciences & Pharmacy, Centre for Brain & Mental Health Research, University of Newcastle. <sup>2</sup>HMRI, Newcastle, Australia.

**AIM:** Stress is thought to play a major role in the pathophysiology of depression. Recent work has implicated dopamine brain circuitry in the underlying mechanisms of depression. The infralimbic (IL) medial prefrontal cortex and nucleus accumbens (NAc) are stress-responsive areas that receive dopaminergic input from ventral tegmental area and are thought to influence susceptibility to depression. Investigation of stress-induced molecular alterations in these areas could help elucidate mechanisms of depression. We carried out gene expression analysis in the IL and NAc of rats submitted to an acute stress paradigm. **METHODS:** Sprague-Dawley rats (n=8/group) were handled and familiarized to an inescapable foot-shock chamber for 4 days. On the 5th day, rats received an electric current passed through the metal grid floor (FS). Shams were treated similarly but did not receive electric current (SHM). Animals were killed 24 hours later and brains removed and processed for microarray based gene expression analysis of the IL and NAc. Genome Studio software was used to identify differentially expressed genes between the groups. **RESULTS:** Our preliminary analysis showed that after normalization, there were 374 genes upregulated and 28 downregulated in IL of FS compared with SHM rats. In contrast, in NAc acute foot-shock resulted in a downregulation of 216 genes and upregulation of just 2 genes. **CONCLUSION:** These results demonstrate that two brain regions involved in the stress response, respond very differently to an acute stressor. By progressively increasing the stress exposure to a chronic state, it will be possible to characterize the molecular changes associated with the manifestation of depression.

## POS-MON-023

# THE EFFECTS OF PHENCYCLIDINE ON THE NMDAR/NEUREGULIN1 SIGNALLING COMPLEX: IMPLICATIONS FOR SCHIZOPHRENIA

Du Bois T.M.<sup>1,2</sup>, Newell K.A.<sup>1,2</sup>, Warren C.R.<sup>1,2</sup> and Huang X.-F.<sup>1,2</sup>

<sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong 2522 NSW, Australia.

<sup>2</sup>Schizophrenia Research Institute, Darlinghurst 2010 NSW, Australia.

Schizophrenia is a devastating disorder affecting 1% of the population worldwide. Phencyclidine (PCP), an N-methyl-D-aspartate receptor (NMDAR) antagonist, is the best known drug that can induce schizophrenia-like symptoms in humans and animals. Using the perinatal PCP animal model, this study investigated the relationship between the NMDAR and neuregulin1 (NRG1) signalling pathways; both are highly implicated in schizophrenia pathology. **Design:** Rats (n=5/group) were treated with PCP (10 mg/kg) or saline on postnatal days (PN) 7, 9 & 11 and were sacrificed on PN12, 35 and 140 for biochemical analyses. Western blotting was used to determine total and phosphorylated levels of NMDAR2A, NMDAR2B, PSD-95 and Akt proteins in prefrontal cortex and hippocampus of PN35 male rats. Levels of NRG1 and its receptor ErbB4 were examined in a parallel study. **Results:** PCP did not affect total or phosphorylated levels of NMDAR2A, NMDAR2B, PSD-95 or Akt in PN35 male rats to a level that reached statistical significance ( $p \geq 0.05$ ). However, alterations in NRG1 and ErbB4 were found in a parallel study at other time-points. These proteins will therefore be further investigated at PN12 and PN140 in both male and female rats. **Discussion:** We have shown in previous studies that perinatal PCP treatment induces long-term alterations in neurotransmitter receptor expression including NMDA and GABA<sub>A</sub>. Levels of other key proteins in the NMDAR and NRG1 signalling pathways will be examined further to determine how these two systems react to brain developmental disruption of NMDAR system, which has implications for schizophrenia aetiology and pathology.

## POS-MON-024

# IMMUNE FACTORS IN ANIMAL MODELS OF SCHIZOPHRENIA

Snikeris P.<sup>1,2</sup>, Huang X.-F.<sup>1,2</sup> and Frank E.<sup>1,2</sup>

<sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia.

<sup>2</sup>Schizophrenia Research Institute, 384 Victoria Street, Darlinghurst, 2010, NSW, Australia.

Schizophrenia is a devastating brain disorder. Whereas its disease mechanisms are still unknown, epidemiological studies show that schizophrenia patients have a lower incidence of inflammatory diseases, indicating a coinciding dysfunction of the immune system. Indeed, various immune factors that are altered in schizophrenia patients, including cytokines, are increasingly shown to play a critical role in various schizophrenia-relevant brain functions. Here, we investigated a panel of schizophrenia-relevant cytokines in two mouse models of schizophrenia. Using a multiplex flow cytometry bead array, IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8(KC), IL-10 and IL-12 were measured in the plasma of Neuregulin-1 knockout (Nrg1 KO) and perinatal phencyclidine (PCP)-treated mice (10mg/kg, ip) at post natal days (PND) 5, 7, 9 and 11 (n=3 each). In the Nrg1 KO mice, TNF- $\alpha$ , IL-2 and IL-8(KC) were up-regulated in a basal state whereas IL-10 showed a trend to be decreased, compared to wild types. Perinatal PCP-treatment had no effect on basal cytokine levels in adult mice (PND56). At PND12, 24 hours after the last treatment, IFN- $\gamma$  and TNF- $\alpha$  levels were decreased in PCP compared to saline treated animals. At weaning (PND21) IL-1 $\alpha$  was decreased in the PCP-treated mice. A similar decrease in IL-1 $\alpha$  was found after exposure to 15min short-term restraint stress in adult mice, independent of the perinatal treatment. The findings in Nrg1 KO mice, indicating a dysregulation of inflammatory cytokines being comparably observed in schizophrenia patients, provide a basis for future translational research into the identification of novel biomarkers and drug targets for schizophrenia as well as the psychopathological potential of a dysregulated neuro-immune system.



## POS-MON-025

## INTRACRANIAL PRESSURE AND OEDEMA IN TWO MODELS OF SUBARACHNOID HAEMORRHAGE

Barry C.M., Van Den Veuvel C., Helps S. and Vink R.  
Discipline of Pathology, School of Medical Sciences, University of Adelaide, Australia.

Subarachnoid haemorrhage (SAH) affects 2000 Australians each year and is usually spontaneous. The average age is 49 and 40% die within 28 days while 30% of survivors have long-term dependency. For the 90% who survive the initial bleed, secondary brain injury affects the brain globally. Secondary injury mechanisms may include raised ICP, brain swelling (oedema) and reduced cerebral perfusion pressure (CPP). In experimental ischaemic stroke, oedema is reduced and functional outcome improved by treatment with the substance P antagonist, n-acetyl tryptophan (NAT). This intervention has not previously been tested in models of SAH. **Methods:** SAH was induced in male Sprague-Dawley rats by either autologous blood injection (prechiasmatic cistern) or arterial puncture (endovascular filament advanced to the Circle of Willis). Sham operated animals acted as controls. SAH animals received treatment with either NAT or vehicle (saline). Functional outcome (neuroscore, n=10/group) and oedema (wet weight-dry weight, n=5/group) were assessed at various time points after SAH. ICP & CPP were monitored before, during & for 5 hours after SAH (n=5/group). **Results:** Haemorrhage volume was larger in puncture SAH animals. Functional deficits were common after puncture SAH and rare after injection SAH. Brain oedema was minimal in both models. ICP increased in both SAH models and was unchanged by administration of NAT. This is consistent with absence of vasogenic oedema. Cerebral perfusion pressure fell below critical levels after puncture SAH. Multiple ICP peaks, representing extremely deleterious events, occurred in 50% of puncture SAH cases. Data indicates that diminished CPP rather than oedema contributes to functional deficits in these models.

## POS-MON-027

GABAA RECEPTOR  $\alpha 3$  CORTICAL EXPRESSION IN PERINATAL ASPHYXIA

Bjorkman S.T.<sup>1</sup>, Miller S.M.<sup>1</sup>, Eckert A.L.<sup>2</sup>, Dodd P.R.<sup>2</sup> and Colditz P.B.<sup>1</sup>  
<sup>1</sup>Centre for Clinical Research, The University of Queensland.  
<sup>2</sup>School of Chemistry and Molecular Biosciences, The University of Queensland.

Perinatal asphyxia is a leading cause of neurodevelopmental delay and death in term neonates. Current therapeutic options are limited to hypothermia and treatment of seizures.  $\gamma$ -aminobutyric acid (GABA) is an inhibitory neurotransmitter in the adult brain however in immature brain GABA can have excitatory actions. Barbiturates and benzodiazepines exert their inhibitory anticonvulsant activity by binding to the GABA<sub>A</sub> receptor. If the GABA<sub>A</sub> receptor is functioning in an excitatory manner when anticonvulsants are administered, drug binding may enhance excitation potentially exacerbating seizures and hypoxic/ischemic brain injury. Efficacy of anticonvulsants in the neonatal brain is not only dependent on the regional and temporal maturation of GABAergic inhibitory function but also on protein expression levels which may be altered following an asphyxial event. Human brain tissue was obtained at autopsy from five control and five asphyxial newborn infants of late gestational age (Queensland Brain Bank). Ethical clearance for the project was obtained under Protocol N° RBH 92/87. Brain tissue from hypoxic/ischemic (H/I) newborn piglet was also obtained. Western blot analysis was used to evaluate the level of expression of the  $\alpha 3$  subunit of the GABA<sub>A</sub> receptor in frontal, motor, temporal and occipital cortex. GABA<sub>A</sub> receptor  $\alpha 3$  protein was elevated in frontal, motor and temporal cortex of the human asphyxial cases when compared to controls. Temporal cortex showed significantly higher  $\alpha 3$  expression ( $p < 0.05$ ) while frontal cortex neared significance ( $p = 0.054$ ); occipital cortex did not alter. We found similar changes in our neonatal H/I piglet with frontal cortex displaying elevated levels of  $\alpha 3$  expression; occipital cortex expression was diminished. Alterations in  $\alpha 3$  subunit expression may influence receptor function and effectiveness of anticonvulsant treatments.

## POS-MON-026

## IMPAIRED EYEBLINK CONDITIONING REVEALS NEURONAL FUNCTION FOR ATM INDEPENDENT OF ITS ROLE IN DNA REPAIR

Birt J.<sup>1</sup>, Lavin M.A.<sup>2</sup>, Luff J.<sup>2</sup>, Power J.<sup>3</sup> and Bellingham M.C.<sup>1</sup>  
<sup>1</sup>School of Biomedical Sciences, University of Queensland, St Lucia, 4072 QLD. <sup>2</sup>Queensland Institute of Medical Research, Royal Brisbane Hospital, 4029 QLD. <sup>3</sup>Queensland Brain Institute, St Lucia, 4072 QLD.

**BACKGROUND:** Ataxia Telangiectasia (AT) is caused by mutation of the PI-3 like protein kinase ATM (Ataxia Telangiectasia Mutated) and results in degeneration of cerebellar Purkinje neurons and cancer. ATM repairs DNA double strand breaks and neurodegeneration is thought to be an oxidative consequence of unstable DNA. **METHODS:** We investigated ATM's neuronal function using cerebellum-dependent delay eyeblink conditioning in vivo and Purkinje synaptic plasticity experiments in vitro, in mice with knock-in deletion of the ATM kinase sequence activated in DNA repair ( $\Delta$ SRI) and mice with complete deletion of ATM (ATM KO). **RESULTS:** Compared to wt mice,  $\Delta$ SRI mice competently acquired a conditioned eyelid response ( $p > 0.05$ , n=11  $\Delta$ SRI, n=11 wt), while ATM KO mice showed reduced acquisition of adaptively timed responses ( $p < 0.05$ ) which were also less robust ( $p < 0.01$ , n=9 ATM KO, 11=wt) by RM ANOVA. Long term synaptic depression in Purkinje neurons was normal in both ATM mutant strains: Average depression was  $18 \pm 0.4\%$  (mean  $\pm$  SEM,  $p < 0.0001$ , n=7,  $\Delta$ SRI),  $18 \pm 0.3\%$  ( $p < 0.0001$ , n=10, wt), and,  $14 \pm 0.6\%$  ( $p < 0.0001$ , n=8, ATM KO)  $13 \pm 0.6\%$ , ( $p < 0.0001$ , n=7 wt); all paired t-test. **CONCLUSIONS:** Mice completely lacking ATM, but not mice with specific deletion of the ATM kinase sequence activated in DNA repair, have a cerebellum-dependent motor deficit, indicating that ATM has a neuronal function independent of its role in DNA repair. As long term depression in Purkinje neurons is intact in both ATM mutants, motor dysfunction must be located downstream from the cerebellar cortex - an unexpected finding given degeneration of Purkinje neurons in human AT.

## POS-MON-028

## FOCAL DAMAGE TO THE ADULT RAT NEOCORTEX INDUCES AXONAL SPROUTING AND DENDRITIC STRUCTURAL PLASTICITY

Blizzard C.A.<sup>1</sup>, Chuckowree J.A.<sup>1</sup>, King A.E.<sup>1</sup>, McCormack G.H.<sup>1</sup>, Chapman J.A.<sup>2</sup>, Vickers J.C.<sup>1</sup> and Dickson T.C.<sup>1</sup>  
<sup>1</sup>Menzies Research Institution, University of Tasmania. <sup>2</sup>School of Medicine, University of Tasmania.

Our ability to effectively manipulate the adaptive response of the brain to injury is limited by a lack of insight into the capacity of the adult CNS for plasticity and remodelling. We have investigated the cellular and architectural alterations following focal brain injury, as well as the specific capacity for structural remodelling of neuronal processes in a subset of cortical interneurons. Focal acute injury was induced by transient insertion of a 25-gauge needle into the neocortex of anaesthetised adult male Hooded-Wistar rats. Animals were perfused at intervals ranging from 1 to 14 days post-injury and brains processed for immunohistochemistry. Focal injury induced proliferation of neural progenitors (nestin-labelled), astrocytes (GFAP-labelled) and microglia (ferritin-labelled). Immunolabelling for BrDU combined with cell-type specific markers confirmed glial, but not neuronal, proliferation. By 7 days post-injury pyramidal neuron markers SMI312 and  $\alpha$ -internexin demonstrated an axonal sprouting response, with fine regenerative sprouts transverse the injury site. By 14 days post-injury, immunohistochemistry confirmed the presence of a dense glial scar at the site of injury. The processes of calretinin-labelled interneurons demonstrated morphological alterations relative to the central microglial/macrophage mass. Quantitative analysis of the dendritic arbor of cells  $> 250 \mu\text{m}$  from the lesion edge demonstrated a significant ( $p < 0.05$ ) change in dendrite polarity, with substantial elaboration distal to the lesion site. There was no significant difference ( $p > 0.05$ ) in mean neurite length or dendrite number for these interneurons. Ultimately, recovery following trauma will require a combination of the induction of new neurogenesis, appropriate regeneration and compensatory plasticity of preexisting pathways. These studies demonstrate that the adult cortex is capable of significant remodeling following brain injury.

## POS-MON-029

**MINOCYCLINE DOES NOT AFFECT NEUROGENESIS, BUT IMPROVES NEUROLOGICAL OUTCOME FOLLOWING TRAUMATIC BRAIN INJURY IN MICE**

Bye N.<sup>1,2</sup>, Ng S.Y.<sup>1,2</sup>, Tran M.<sup>3</sup>, Semple B.D.<sup>1,2</sup>, Kossmann T.<sup>4</sup> and Morganti-Kossmann M.C.<sup>1,2</sup>

<sup>1</sup>National Trauma Research Institute, Alfred Hospital, Melbourne, Australia. <sup>2</sup>Department of Medicine, Monash University, Australia. <sup>3</sup>Department of Physiology, University of Melbourne. <sup>4</sup>The Epworth Hospital, Richmond, Australia.

Neurogenesis is stimulated following brain injury and potentially contributes to tissue repair; however, this response may be limited by elevated levels of inflammatory cytokines. Therefore, we investigated whether treatment with the anti-inflammatory drug minocycline could attenuate inflammation, enhance specific stages of neurogenesis, and improve neurological outcome in a closed head injury (CHI) model of focal traumatic brain injury (TBI). Adult C57BL/6 mice were treated as: CHI+minocycline (d1: 45mg/kg, d2-7or14: 22.5mg/kg, i.p. twice/day), CHI+vehicle, and sham-operated controls. BrdU (d1-4: 200mg/kg i.p.) was administered to label proliferating cells. Neurological outcome was assessed, and brains were collected at 1&6w (n=6-7). BrdU- and DCX-immunolabelled cells were quantified in the dentate gyrus (DG) and subventricular zone (SVZ), to assess cell proliferation/survival and neuronal differentiation, respectively. Neuronal and glial maturation/survival at 6w in the DG and pericontusional cortex was determined by BrdU co-labelling with NeuN and GFAP. Minocycline reduced neurological dysfunction from 24h to 6w following trauma ( $p < 0.05$  vs. vehicle-controls), and tended to decrease F4/80+ microglia at 1w ( $P = 0.083$ ). While BrdU-labelled and Dcx-labelled cells were increased in the SVZ and DG of traumatised mice at 1&6w compared to shams ( $P < 0.05$ ), no differences were observed between minocycline and vehicle groups ( $P > 0.05$ ). Also, the percentages of new neurons and astrocytes at 6w post-injury were not different with minocycline treatment ( $P > 0.05$ ). This study demonstrates that minocycline does not affect precursor proliferation, neuronal differentiation, or new cell survival after experimental TBI. However, minocycline-treatment was associated with improved neurological outcome, which may be due to its anti-inflammatory actions.

## POS-MON-030

**CYTOKINE EXPRESSION IN POST MORTEM HUMAN BRAIN TISSUE FOLLOWING ACUTE TRAUMATIC BRAIN INJURY**

Frugier T.<sup>1</sup>, Mclean C.A.<sup>2</sup>, O'Reilly D.<sup>1</sup> and Morganti-Kossmann M.C.<sup>1</sup>

<sup>1</sup>National Trauma Research Institute, Melbourne, VIC. <sup>2</sup>Anatomical Pathology Department, The Alfred Hospital, Melbourne, VIC.

**Introduction:** Little is known about the molecular events following severe traumatic brain injury (TBI) in humans and to date there are no efficient therapies. The availability of human brain tissue from the Australian Neurotrauma Tissue Bank is a unique opportunity to analyse the early inflammation following TBI. **Methods:** A total of 21 trauma brain samples and 13 age/sex matched control samples were investigated. We analysed 9 inflammatory cytokines at mRNA and protein level using bioplex-assay and quantitative-PCR. Axonal pathology was studied using immunohistochemistry against APP and Neurofilament-200kD proteins.

**Results:** All the pro-inflammatory mediators analysed showed a strong and significant ( $p < 0.001$ ) increase in the brain samples of individuals who died more than 6 hours following injury. In the brain samples of individuals who died within 17 minutes of injury, IL-6 ( $p < 0.027$ ), IFN- $\gamma$  ( $p < 0.018$ ), TNF- $\alpha$  ( $p < 0.03$ ) and GM-CSF ( $p < 0.022$ ) concentrations were already found increased. However, the anti-inflammatory cytokines IL-4 and IL-10 levels remained unchanged. Similarly, quantitative-PCR showed that IL-6, IL-1 $\beta$ , IL-8 and TNF- $\alpha$  mRNA levels were increased ( $p < 0.001$ ) more than 6 hours after injury, with TNF- $\alpha$  showing an increase within 17 minutes of the injury ( $p < 0.014$ ). No statistical difference was observed between the damaged and the contralateral cortex. Finally, in all the cases with a survival time of 8 hours or longer, numerous damaged axons were detected, indicating that diffuse brain injury was present.

**Conclusions:** This study shows clearly for the first time in human brain tissue that i) the inflammatory response begins immediately after the traumatic impact; ii) diffuse secondary axonal injury may contribute to the extent of cellular and humoral neuroinflammation; and iii) cytokines/chemokines detected in the brain tissue are produced locally by intraparenchymal cells in the early stages of the inflammatory cascade and do not diffuse from the systemic circulation.

## POS-MON-031

**DOWN-REGULATION OF THE SEROTONIN TRANSPORTER FOLLOWING PRETERM HYPOXIC-ISCHEMIC BRAIN INJURY**

Wixey J.A., Reinebrant H.E. and Buller K.M.

Perinatal Research Centre, Clinical Neuroscience, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, Herston, 4029, QLD.

**Purpose:** Serotonin (5-HT) plays a key role in the regulation of numerous cognitive, motor and behavioural functions. The serotonin transporter (SERT) is the most critical regulator of 5-HT as it terminates serotonergic signalling by the reuptake of extracellular 5-HT. Whether a hypoxic-ischemic (HI) event in the preterm neonate affects serotonin or SERT levels in the brain is unknown. We hypothesised that neonatal HI can alter serotonin and SERT expression in the neonatal brain and examined whether modulating neuroinflammation can alleviate this injury. **Methods:** Using a P3 Sprague-Dawley HI rat pup model (right carotid ligation + 30 min 6% O<sub>2</sub>) and human neonatal brain tissue, we examined the effect of HI on serotonin levels and SERT expression in brains of control (rat P4 n=7; P10 n=9; P45 n=9; human n=3) and HI (rat P4 n=9; P10 n=10; P45 n=10; human n=3) rat pups and human neonates. We also determined whether blocking activated microglia (minocycline 45 mg/kg) altered serotonin levels and SERT expression in control (P10 n=8; P45 n=7) and HI (P10 n=10; P45 n=9) rat pups. **Results:** Forebrain serotonin and SERT expression decreased in the P3 HI rat brain and human HI neonatal brain. Density and morphologic changes in SERT immunolabelling were apparent one and six weeks after P3 HI. Minocycline treatment attenuated the decrease in serotonin levels and SERT expression one week post insult. **Conclusion:** The serotonergic system is affected by neonatal HI and this may contribute to long-term neurological deficits in the HI neonate. Furthermore, minocycline may have neuroprotective actions after neonatal brain injury via mechanisms involving modulation of serotonergic networks in the central nervous system.

## POS-MON-032

**ENDOGENOUS BRAIN ALLOPREGNANOLONE IS INCREASED FOLLOWING EXOGENOUS ADMINISTRATION OF ALLOPREGNANOLONE IN AN ANIMAL MODEL OF STROKE**

Tomkins A.J., Calford M.B. and Spratt N.J.

School of Biomedical Sciences and Hunter Medical Research Institute, Faculty of Health, University of Newcastle, Callaghan, NSW 2308, Australia.

Allopregnanolone, a progesterone metabolite, is the most potent known modulator of the GABA<sub>A</sub> receptor enhancing GABA-mediated inhibition in the brain. Allopregnanolone has been studied as a potential neuroprotective treatment following stroke and other excitotoxic diseases. Endogenous brain levels of allopregnanolone have been shown to increase in models of excitotoxicity however the endogenous response to stroke is not known. This pilot study aimed to quantify endogenous levels of allopregnanolone in an animal of stroke following exogenous allopregnanolone treatment. Spontaneously hypertensive rats (SHR) (n=7) underwent 90 minutes of middle cerebral artery occlusion (MCAo) by intraluminal thread-occlusion. After vessel reperfusion, animals received treatment of allopregnanolone (8mg/kg, n=3) administered intraperitoneally 110 minutes post-occlusion. Controls received no treatment (n=4). Tissue samples were taken 1 hour post-injection from core and penumbra regions of ipsilateral hemisphere and homotypic regions of contralateral. Steroid extraction was achieved by methanol extraction followed by C18 solid phase extraction. Steroid extracts were derivatized using the silylation reagent BSTFA + TMCS (99:1). Steroid analysis and quantification was performed using a quadrupole GCMS system with electron-impact ionisation. Whole brain levels of allopregnanolone were significantly higher in injection animals ( $171.7 \pm 43$  ng/mg) compared to non-injection controls ( $66.8 \pm 31$  ng/mg). Individual brain regions (core and penumbra) were also significantly higher in both hemispheres of injection animals compared to non-injection controls. This preliminary study indicates that exogenous allopregnanolone treatment is appropriate for increasing endogenous levels in whole brain including non-perfused stroke regions.

## POS-MON-033

**CHANGES IN GFAP PHOSPHORYLATION IN THE HYPOXIC/ISCHEMIC BRAIN**

**Sullivan S.M.**, Bjorkman S.T., Miller S.M. and Colditz P.B.  
Centre for Clinical Research, The University of Queensland, Herston, QLD, 4029.

Hypoxic/ischemic (H/I) brain damage is a leading cause of death and neurodevelopmental disability in neonates. Our previous research has suggested that astrocytes are important in determining the survival of neurons and thus the biology of astrocytes can influence overall outcomes after H/I brain damage. The astrocytic cytoskeletal protein glial fibrillary acidic protein (GFAP) can be phosphorylated at multiple sites by various enzymes. Phosphorylation shifts the equilibrium from the polymeric form to the less stable monomeric form of the protein. We have investigated whether the phosphorylation state of GFAP is altered after an H/I insult. Neonatal pigs (N=10) were anaesthetised and exposed to 4% oxygen for 30min, including 10min of ischemia. Control littermates (N=5) were exposed to anaesthesia, but not the H/I insult. Pigs were allowed to recover for 72hr, were euthanased and brain tissues removed. Slices from the left hemisphere were frozen for molecular/protein analyses and slices from the right hemisphere were fixed in paraformaldehyde for histology and immunohistochemistry. Polyclonal antibodies were generated in rabbits against the phosphorylated form of GFAP (pGFAP). Dot blots revealed that the antibodies specifically detected pGFAP and Western blots revealed a band (~50kDa), corresponding to the predicted molecular weight of the protein. Western blot analysis revealed a significant increase in pGFAP in the cortex of H/I animals ( $P<0.05$ ) compared to controls. Immunohistochemical studies will determine whether increased pGFAP alters astrocyte morphology, which would influence neuronal survival after H/I insults. Future studies will examine whether therapies targeted at preventing or reversing GFAP phosphorylation offer an alternative pathway to neuroprotection in the neonatal H/I brain.

## POS-MON-035

**POST-TRAUMATIC HYPOXIA EXACERBATES NEUROLOGICAL DEFICITS, NEUROINFLAMMATION, AND AXONAL DAMAGE FOLLOWING TRAUMATIC AXONAL INJURY**

**Hellewell S.C.<sup>1,2</sup>**, Yan E.B.<sup>1,2</sup>, Agyapomaa D.A.<sup>1,2</sup> and Morganti-Kossmann M.C.<sup>1,2</sup>

<sup>1</sup>National Trauma Research Institute, Melbourne. <sup>2</sup>Central Clinical School, Monash University, Melbourne.

Post-traumatic hypoxia is common in severe TBI patients, with worsened neurological outcomes. In this study, we explored whether post-traumatic hypoxia exacerbates neurological deficit, neuroinflammation, glial activation, and axonal damage. Methods: Diffuse traumatic axonal injury (TAI) was produced by dropping a 450g weight from 2m. An additional hypoxic insult was induced by ventilation with 14% O<sub>2</sub> in N<sub>2</sub> for 30min after TAI. Results: TAI+hypoxia animals showed severe neurological deficit on the Rotarod (1d: 3.4±1.6 rpm; 6d: 12.8±2.8) than TAI+normoxia rats (1d: 8.2±2.1; 6d: 18.8±2.5  $P<0.05$ ). CD68-positive cells were localised primarily in the corpus callosum and optic tract with a significant increase in TAI+hypoxia rats (19.5±7.4 cells/region; 216.6±30.0, respectively) over TAI+normoxia (6.7±3.0; 142.4±9.6) or sham animals (0.5±0.3; 4.9±4.9) ( $P<0.05$ ). TAI+hypoxia rats showed a significant increase in IL-6 (12.7±2.0 pg/mg protein) and IL-1 $\beta$  (2.4±0.2) concentrations in the brain homogenates at 1d when compared with TAI+normoxia (IL-6: 8.3±0.6; IL-1 $\beta$ : 1.8±0.1  $P<0.05$ ). Amyloid precursor protein (APP) staining showed a significant increase in the numbers of retraction bulbs in the corpus callosum of TAI+hypoxia rats (69.0±18.6 bulbs/region) when compared with TAI+normoxia (38.5±28.22) at 1d. Furthermore, in the corpus callosum a significant increase in swollen axons was evident in the TAI+hypoxia rats (50.3±45.7) compared with the TAI+normoxia rats (24.0±10.2) at 1d. These results suggest hypoxia exacerbates neurological deficits after TAI, worsens neurological outcome and perpetuates secondary injury mechanisms, including neuroinflammation, glial activation and axonal damage.

## POS-MON-034

**A NOVEL MIMETIC PEPTIDE AGAINST CONNEXIN43 ENHANCES NEURONAL SURVIVAL IN A RAT MODEL OF SPINAL CORD CONTUSION INJURY**

**Gorrie C.A.<sup>1</sup>**, Roberts S.<sup>1</sup>, Waite P.M.E.<sup>1</sup>, Nicholson L.F.B.<sup>2,3</sup>, Green C.R.<sup>4</sup> and O'Carroll S.J.<sup>2,3</sup>

<sup>1</sup>Neural Injury Research Unit, University of New South Wales.

<sup>2</sup>Centre for Brain Research, University of Auckland. <sup>3</sup>Department of Anatomy with Radiology, University of Auckland. <sup>4</sup>Department of Ophthalmology, University of Auckland.

Introduction: Connexin43 is a gap junction protein that is up-regulated after SCI leading to lesion spread. We have previously shown that application of connexin43 mimetic peptide caused a decrease in tissue swelling, a reduction in astrocytosis and promoted neuronal cell survival in spinal cord explants<sup>1</sup>. In vivo studies using a rodent spinal cord contusion model demonstrated a transient improvement in locomotor scores, reductions in lesion size ( $p<0.05$ ) and reduced GFAP staining intensity at 6 weeks post injury<sup>2</sup>. In the current study we used western blot analysis to further investigate the effect of mimetic peptide treatment on astrogliosis and neuronal survival. Methods: Rats (n=32) were subjected to a 10g, 12.5 mm weight drop injury at the vertebral level T10. An intrathecal catheter attached to an Azlet osmotic pump was used to deliver vehicle or connexin43 peptide (5, 20 or 50  $\mu\text{mol/kg}$ ) to the lesion site at a rate of 8 $\mu\text{l/hr}$  for 24 hours. Animals were killed at 6 weeks post injury and tissue collected. Results: Western blot analysis confirmed the decrease in GFAP protein levels ( $p<0.05$ ) and demonstrated a significant increase ( $p<0.05$ ) in the levels of the proteins NeuN, a marker for mature neurons and the neurofilament marker SMI-32 in the 5  $\mu\text{mol/kg}$  treatment group compared with controls. Conclusions: These results further indicate the potential for connexin43 channel modulation using mimetic peptides to improve outcomes following spinal cord injury. 1 O'Carroll, S et al (2008) Cell Communication & Adhesion, 15:1,27–42 2. Gorrie, CA et al (2009) Proc. Aust. Neuroscience Soc. Vol 19.

## POS-MON-036

**BEHAVIOURAL DEFICITS IN RATS FOLLOWING ISCHEMIC STROKE – A LONGITUDINAL STUDY**

**Rewell S.S.<sup>1,2</sup>**, Porritt M.J.<sup>1,2</sup> and Howells D.W.<sup>1,2</sup>

<sup>1</sup>Department of Medicine (Austin Health), University of Melbourne.

<sup>2</sup>Florey Neuroscience Institutes; Heidelberg, Victoria, Australia.

Few experimental stroke studies extend survival time beyond a week post stroke. Using our optimised model of rat Middle Cerebral Artery thread occlusion, we aimed to evaluate the usefulness of behavioural tests in detecting long term deficits out to 24 weeks post stroke. 105 male Spontaneously Hypertensive Rats were randomly allocated to one of nine groups. Stroke animals underwent 90 minute transient MCAo while the sham group underwent identical surgery without thread insertion. The surgery was followed by a range of recovery times: 24 hours, 3, 7, 14, 21, 28 days, 12 and 24 weeks (n $\geq$ 11 per group). Neurological deficit was assessed at each time point (and additionally at 8, 16 and 20 weeks) using three behavioural tests: a basic behavioural deficit (assessment of reflex and mobility); a modified sunflower seed task (fine motor skill) and an adhesive sticky tape removal test (sensory neglect and motor skill). Basic behavioural deficit generally resolved within 14 days, of which forelimb flexion was the most affected. A simplified analysis of the sunflower seed task, which involved counting untouched seeds and the number of broken seed pieces, failed to show differences between stroke and sham animals beyond 14 days. The sticky tape test highlighted continual neglect of the contralateral forepaw both in the acute period and for the 24 weeks following stroke. Of the three behavioural tests evaluated, the sticky tape test shows most promise in detecting long term deficits in stroke animals. Further analysis of behavioural change over time, together with histological examination will provide insights into the development of damage and process of behavioural recovery.



## POS-MON-037

**CONFOUNDING NEURODEGENERATIVE EFFECTS OF MANGANESE FOR IN-VIVO MR IMAGING IN RAT MODELS OF BRAIN INSULTS**

Cardamone L.<sup>1</sup>, Bouilleret V.<sup>1,2</sup>, Liu Y.R.<sup>1</sup>, Koe A.S.<sup>1</sup>, Fang K.<sup>3</sup>, Williams J.P.<sup>3</sup>, Myers D.E.<sup>1</sup>, Jones N.C.<sup>1</sup> and O'Brien T.J.<sup>1,4</sup>

<sup>1</sup>Department of Medicine (RMH), University of Melbourne, Parkville, Victoria, Australia, 3052. <sup>2</sup>Department of Neurophysiology and Epilepsy, ApHp, CHU Bicetre, Paris, France, 94275. <sup>3</sup>Small Animal MRI Facility, Florey Neurosciences Institute, Parkville, Victoria, Australia, 3052. <sup>4</sup>Department of Neurology (RMH), University of Melbourne, Victoria, Australia, 3052.

Manganese-enhanced magnetic resonance imaging (MEMRI) is an emerging technique to visualize structural and functional detail of the brain in-vivo in experimental animal models of neurological disease or injury. However, the potential for Mn<sup>2+</sup> to cause cellular toxicity which could confound the experimental effect is often overlooked. In this study, we examine long-term consequences of manganese exposure in the fluid-percussion injury (FPI) model of closed head injury. Two groups of adult male Wistar rats (n=72 in total) were studied with either Mn<sup>2+</sup>-enhanced MRI (MEMRI), whereby rats receive MnCl<sub>2</sub> (100mg/kg i.p.) 24 hours prior to scanning, or standard MRI (sMRI) with no contrast agent. Rats from both groups underwent either FPI or sham injury, and were longitudinally assessed up to 6 months for signs of neurological toxicity using behavioural tests, a stress responsivity assay, EEG recording and MRI scanning. Animals in the MEMRI group, regardless of injury status, showed dramatic and progressive signs of cerebral toxicity, evidenced by significantly reduced weight gain (p<0.0001); progressive brain volume decrease (p<0.0001); significantly increased anxiety- (p<0.0001) and depressive- (p=0.026) like behaviours; and significantly enhanced stress responsivity (p=0.044), compared with rats in the sMRI group. These outcomes were compounded by the effect of neurotrauma. These results demonstrate long-term structural and functional consequences of the use of manganese as a contrast agent for in-vivo MRI in rats. These consequences which can confound experimental outcomes must be taken into account when designing longitudinal imaging studies using manganese-enhanced MRI.

## POS-MON-039

**INTERLEUKIN-17 CONTRIBUTES TO NEUROINFLAMMATION AND NEUROPATHIC PAIN FOLLOWING PERIPHERAL NERVE INJURY**

Kim C. and Moalem-Taylor G.

School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia.

Cytokines, essential mediators of inflammatory and immune responses, play an important role in the pathophysiological processes associated with neuropathic pain following peripheral nerve injury. Recently, a novel proinflammatory cytokine, the interleukin (IL)-17, was found to orchestrate inflammatory responses in a wide range of inflammatory and autoimmune diseases of the nervous system. Here, we investigated the role of IL-17 in mediating neuroinflammation and pain hypersensitivity using the neuropathic pain model of partial ligation of the sciatic nerve in mice. Compared to wild-type, IL-17 knockout mice displayed significantly decreased mechanical pain hypersensitivity (n= 6 mice per group) as well as decreased infiltration of T cells and macrophages to the injured sciatic nerves and the L3-L5 dorsal root ganglia and decreased activation of microglia and astrocytes in the L3-5 dorsal and ventral horns of the spinal cord (n= 3-4 mice per group). Further, intraplantar and intraneural injection of recombinant IL-17 into the hind-paw and the sciatic nerve, respectively, induced both mechanical allodynia and thermal hyperalgesia, while intrathecal injection produced thermal hyperalgesia (n=6 mice per group). Taken together, our results demonstrate that IL-17 contributes to the regulation of immune cell infiltration and glial activation after peripheral nerve injury and the ensuing neuropathic pain.

## POS-MON-038

**INHIBITION OF NEUROINFLAMMATION PREVENTS RAPHE NEURON LOSS AFTER HYPOXIC-ISCHEMIC BRAIN INJURY**

Reinebrant H.E., Wixey J.A. and Buller K.M.

Perinatal Research, Clinical Neuroscience, University of Queensland Centre for Clinical Research, Brisbane, QLD 4029, Australia.

Neuroinflammation plays a key role in the generation of brain injury after neonatal hypoxia-ischemia (HI). However it is not clear whether brainstem serotonergic raphe neurons are lost after neonatal HI and indeed if neuroinflammation influences the demise of serotonergic neurons. The rate-limiting step in serotonin synthesis involves tryptophan hydroxylase (TpH) and the actions of 5HT are terminated by re-uptake of 5HT via the serotonin transporter (SERT). We used a postnatal day 3 (P3) HI rat model (right common carotid occlusion + 30 min 6% O<sub>2</sub>) to determine the effects of P3 HI on the brainstem serotonergic system one (P10) and six (P45) weeks after HI. In addition, we examined the effects of minocycline administration, a potent inhibitor of neuroinflammation (45 mg/kg P3 HI, 22.5 mg/kg P4 to P9). Using immunolabelling to identify 5HT- and SERT-positive neurons and Western blotting to determine SERT and TpH protein levels, we examined the effects of P3 HI and minocycline treatment on the serotonergic system (n>5 per group). We found after P3 HI, there was a significant loss of 5HT-positive neurons and TpH protein levels in the dorsal raphe on P10 and P45 compared to control animals. In addition, the SERT protein expression was significantly down-regulated on P10, but not on P45 compared to control animals. Minocycline treatment prevented the neuronal loss and decreases in TpH and SERT on P10 but not on P45. We conclude that the 5HT system in the brainstem is disrupted after neonatal HI and that minocycline could be a potential therapeutic intervention to block neuroinflammation and prevent damage to the brainstem serotonergic system.

## POS-MON-040

**EVIDENCE OF APOPTOSIS IN THE PERIAQUEDUCTAL GREY OF RATS WITH DISABILITY AND PAIN AFTER PERIPHERAL NERVE INJURY**

Mor D. and Keay K.A.

School of Medical Sciences (Anatomy & Histology), University of Sydney, NSW, Australia.

Constriction injury of the sciatic nerve (CCI) results in hyperalgesia and allodynia (*pain*) in all rats. In contrast, we have shown that only 30% of nerve-injured rats develop *disabilities* (i.e., altered social behaviours, sleep, affect). These *disabilities* resemble those of human neuropathic pain patients. The *disabled* rats also show select neural (mal-) adaptations in the midbrain periaqueductal grey (PAG). In particular, there is increased expression of GFAP mRNA and protein, in the lateral and ventrolateral columns of this region. These markers reveal significant activation of astrocytes in the PAG, which suggests significant cellular damage in this region. To evaluate this further, additional markers of cellular damage were investigated in the isolated PAG of *disabled* (N=8) versus *non-disabled* (N=8) rats. Using RT-PCR the expression of Bax; bcl2; Heat Shock Protein 60 (HSP60) and iNOS expression was probed. Bax showed a select increase (1.145 fold) in *disabled* rats, while bcl-2 expression showed a significant down-regulation in both groups (-0.712 in *disabled* rats). The Bax/bcl2 ratio was raised in both groups, however the highest ratio (1.54) was found in rats with *disabled*. Further, HSP60 was significantly down-regulated, and iNOS was significantly up-regulated, in *disabled* rats (0.83 and 2.298 respectively). Finally, TUNEL labelling was used to quantify apoptosis at day 6, post-injury in histological sections from *disabled* (N=8) versus *non-disabled* (N=8) rats. TUNEL positive nuclei were found in the lateral and ventrolateral, PAG of *disabled* rats, the numbers of TUNEL profiles in the vPAG correlated significantly with the degree of disability. These data suggest that the disabilities expressed by a subpopulation of nerve-injured rats may result from neuronal cell loss in the lateral and ventrolateral PAG, and the parallel neural networks in which they sit.



## POS-MON-041

**EFFECTS OF SILENCING CONNEXIN-43 EXPRESSION ON THE ASTROCYTIC RESPONSE TO INJURY IN CELL CULTURES**

Homkajorn B., Sims N.R. and Muyderman H.

Centre for Neuroscience, Flinders Medical Science and Technology, School of Medicine, Flinders Medical Centre, Flinders University, Adelaide.

Connexin-43 (Cx43) is the major component of gap junctions in astrocytes and has been implicated as a possible contributor in the response of these cells to tissue damage. This study directly tested the involvement of Cx43 in the response to injury of astrocytes in primary culture. **Methods:** Astrocytes were transfected using nucleofection with a plasmid encoding both Green Fluorescent Protein (GFP) and interfering RNA directed against Cx43. The consequences of Cx43 knockdown for recovery after scratch wound injury were assessed. **Results:** Transfection efficiency was  $62 \pm 12\%$  ( $n=10$ ). The GFP-positive cells showed greatly reduced immunoreactivity for Cx43 ( $n=3$ ). Western blots indicated essentially complete depletion of Cx43 in the transfected cells ( $n=3$ ). Consistent with this observation, analysis of Fluorescence Recovery after Photobleaching revealed greater than 70% reduction in cell connectivity compared with non-transfected cells ( $n=3$ ). Cultures containing cells with reduced Cx43 expression showed a similar rate of recovery from scratch injury compared with control cells that had been transfected with DNA-encoding GFP only ( $n=4$ ). The total number of astrocytes and the proportion of transfected cells within the recovering wound were also similar for the two preparations at 24 and 72 h after the scratch. However, the contribution of the transfected cells to the wound closure at 72 h (but not 24 h) was less for cultures with reduced Cx43 expression compared with the GFP-only controls. Thus, Cx43 had no obvious role in the initial response to injury but apparently contributed to subsequent cell migration or process outgrowth that was involved in further closure of the scratch wound.

## POS-MON-043

**HYPOTHERMIA DURING TRANSIENT FOCAL ISCHAEMIA IN SPONTANEOUSLY HYPERTENSIVE RATS IS NOT NEUROPROTECTIVE**McLeod D., Tomkins A., Pepperall D., Chung S., Calford M. and Spratt N.  
University of Newcastle.

Moderate hypothermia (28 °C) for 2 hours has been shown to reduce infarct volume in spontaneously hypertensive rats (SHR) following transient focal ischaemia using an intracerebral middle cerebral artery (MCA) occlusion method, and to markedly reduce cerebral oedema even 3 days later. The present study sought to determine whether systemic cooling to 32-33 °C provides neuroprotection in SHR when initiated from the onset of transient MCA occlusion (MCAo) using the thread-occlusion technique. A total of 18 rats were used in the study. Rats were subjected to neurological tests (sticky dot and tape removal tests) before and after stroke treatment. Under isoflurane anaesthesia, all rats underwent 90 minute MCAo and were implanted with intra-abdominal temperature dataloggers. The normothermic and hypothermic animals ( $n=9$  each) were maintained at 37 °C and 32.5 °C ( $\pm 0.5$  °C) respectively for 2 hours. Neurological tests were done at 4, 24, 48 and 72 hours following MCAo. All rats were euthanized at 72 hours. There was no difference between normothermic and hypothermic groups for infarct volumes ( $87 \pm 45$  v.  $106 \pm 27$  mm<sup>3</sup>, NS) or neurological scores at any time point, but, there was significant reduction in oedema volume ( $43 \pm 20$  v.  $25 \pm 20$  mm<sup>3</sup>,  $P < 0.05$ ). Following rewarming of hypothermic animals, there were no body temperature differences between groups over 72 hours. During anaesthesia there were no differences between groups for blood pressure or SpO<sub>2</sub>. However respiratory rate and HR were significantly lower in the hypothermia group at specific time points during the cooling period. In summary, hypothermia did not provide neuroprotection in SHR following transient thread occlusion of the MCA but did prevent oedema formation even 3 days later. Potential reasons for the discrepancy with previous results will be discussed.

## POS-MON-042

**IDENTIFICATION OF CELLULAR RESPONSES TO AUTOIMMUNE INJURY IN NEURONS**Jonas A.<sup>1,2</sup>, Gresle M.<sup>1,2</sup>, Perreau V.<sup>2</sup>, Kilpatrick T.<sup>1,2</sup> and Butzkueven H.<sup>1,2</sup><sup>1</sup>Multiple Sclerosis Group, Florey Neuroscience Institutes, Howard Florey Institute, Parkville, Victoria 3010, Australia. <sup>2</sup>Centre for Neuroscience, The University of Melbourne, Parkville, Victoria 3010, Australia.

In multiple sclerosis (MS) axonal/neuronal injury appears to play an important role in early disease activity, and is regarded as the underlying cause of permanent disability. There is a current need therefore, to identify novel targets for neuroprotection in MS. We aimed to identify endogenous genes that could limit axonal/neuronal injury in the context of neuro-inflammatory disease. An unbiased comparative microarray analysis of gene expression was conducted on motor cortex enriched tissue from mice subjected to the autoimmune disease experimental autoimmune encephalomyelitis (EAE) ( $n = 6$ ), relative to healthy, unchallenged controls ( $n = 4$ ). Although spinal cord and optic nerves are the main sites of damage in this model, we chose to examine motor cortex tissue to facilitate the detection of genes regulated in neurons, rather than inflammatory cells. Importantly, cortico-spinal motor neurons project axons from the motor cortex to the spinal cord, and are, therefore, associated with, but distant to inflammatory foci. Using this strategy, we identified 76 genes that were significantly regulated in EAE mice ( $p < 0.05$ ). Our initial results indicate that a group of genes that play a role in the modification and production of the extracellular matrix are highly regulated in this disease. Some of these genes include Fibronectin, Pappalysin-2, Von Willebrand factor and Plakophilin-2. Changes in the expression of these genes were validated using RT-PCR. It is hoped that this experimental approach will allow us to identify endogenous genes that limit axonal/neuronal injury, and provide novel therapeutic targets for neuroprotection in MS.

## POS-MON-044

**ABLATION OF INSULIN-REGULATED AMINOPEPTIDASE GENE PROTECTS AGAINST ISCHEMIC DAMAGE IN THE BRAIN**Pham V.<sup>1</sup>, Downes C.E.<sup>2</sup>, Wong C.H.Y.<sup>2</sup>, Diwakarla S.<sup>1</sup>, Albiston A.L.<sup>1</sup>, Ng L.<sup>1</sup>, Lee S.<sup>1</sup>, Crack P.J.<sup>1</sup> and Chai S.Y.<sup>2</sup><sup>1</sup>Florey Neuroscience Institutes, <sup>2</sup>Department of Pharmacology, University of Melbourne, Vic 3010, Australia.

Insulin-regulated aminopeptidase (IRAP) is a zinc-dependent transmembrane metallopeptidase that degrades small neuropeptides including vasopressin, oxytocin, enkephalins, CCK8 and somatostatin and is also involved in the trafficking of the insulin-responsive glucose transporter-4 (GLUT-4) vesicles. In the brain, IRAP is found predominantly in neurons, with high concentrations occurring in pyramidal neurons in the cortex and hippocampus. IRAP was found upregulated in activated astrocytes and microglial following damage. The present study investigated the role of IRAP in ischemic stroke using the model of middle cerebral artery (MCA) occlusion on wildtype and IRAP knockout mice. A significant 80% reduction in infarct volume was observed in the IRAP knockout mice ( $n=8$ ) compared with that of wild-type littermates ( $n=13$ ) after a 2-h occlusion of MCA followed by reperfusion with an associated significant improvement in neurological function. Cerebral blood flow, as measured by the laser Doppler, was partially restored in the ischemic hemisphere throughout the 2-h occlusion of MCA in the IRAP knockout mice. The increase in the cerebral blood flow was not due to the difference in the anatomy of circle of Willis (using the Evans blue staining) or microvessel density (using immunofluorescent staining for the endothelial-specific marker CD31) between IRAP knockout and wildtype mice. Western blot analysis of the ischemic brain cortices of both wildtype and IRAP knockout mice ( $n=5-7$ ) revealed similar levels of the expression of phospho-eNOS, nNOS and iNOS proteins. The data in this current study has indicated that deletion of IRAP gene has a protective effect on ischemic brain injury, at least partially through the modulation of collateral blood flow.

## POS-MON-045

**SPINAL CORD COMPRESSION INJURY – THE ROLES OF EARLY DECOMPRESSION AND HYPOTHERMIA IN FUNCTIONAL RECOVERY**

**Kerr N.F.<sup>1</sup>**, Gatt A.M.<sup>1</sup>, Ghasem-Zadeh A.<sup>2</sup>, Aleksoska E.<sup>1</sup>, Cox S.F.<sup>1</sup>, Wills T.E.<sup>1</sup>, Howells D.W.<sup>1</sup> and Batchelor P.E.<sup>1</sup>

<sup>1</sup>The University of Melbourne, Department of Medicine, Austin Health.  
<sup>2</sup>Endocrinology Centre of Excellence, Austin Health.

**Purpose:** Spinal cord compression occurs in the majority of traumatic spinal cord injuries (SCI). Currently, patient stabilisation and difficulties in organising early surgery mean that decompressive surgery is performed relatively late. Persistent compression is not routinely modelled in animal SCI, however decompression is reported to provide functional recovery (Dimar et al, 1999). In this study we investigated the impact of hypothermia and early decompression on functional motor and tissue outcomes following traumatic SCI. **Methods:** 12-16 week female F344 rats (n = 72) were subject to a moderate spinal cord contusion (150Kdyne) at T7-9. Epoxy spacers were inserted immediately after injury to compress the spinal cord by 45%. Decompression was performed either immediately, 2hrs or 8hrs post-injury. Half were treated with hypothermia (33°C) commencing 30mins post-injury, maintained for 7.5hrs, with the other half remaining normothermic (37.4°C) for the same period. Functional motor recovery was assessed over 8 weeks by the BBB score (Basso et al, 1996) and the ladder stepping test. Overall tissue damage was assessed on H&E-stained sections. **Results:** Hypothermia significantly improved behavioural and histological outcomes in the 8hr compression group. The hypothermics regained weight-supported locomotion while the normothermics remained severely paraparetic. Trends in favour of hypothermia were seen in behavioural and histological outcomes of the immediate and 2hrs decompression cohorts. Overall, the data demonstrates increasing relative benefit of hypothermia with increasing duration of compression. **Conclusion:** In a model of SCI that replicates severe compression following initial trauma, hypothermia is of significant benefit. The data indicate that hypothermia would be a useful therapy to prevent neurological decline prior to decompressive surgery.

## POS-MON-046

**DEFICIENCY OF THE CHEMOKINE RECEPTOR CXCR2 ATTENUATES NEUTROPHIL INFILTRATION AND LESION VOLUME FOLLOWING CLOSED HEAD INJURY**

**Semple B.D.<sup>1,2</sup>**, Bye N.<sup>1,2</sup>, Ziebell J.M.<sup>1</sup> and Morganti-Kossmann M.C.<sup>1,2</sup>

<sup>1</sup>National Trauma Research Institute, Alfred Hospital, Melbourne, Australia. <sup>2</sup>Department of Medicine, Monash University, Melbourne, Australia.

CXCR2 is the principle receptor through which the chemokines CXCL1 (KC), CXCL2 (MIP-2) and CXCL8 (IL-8) induce neutrophil migration. Upregulated following traumatic brain injury, these chemokines likely contribute to neutrophil infiltration and secondary brain damage. Thus we investigated the consequences of CXCR2 deficiency in a mouse focal closed head injury (CHI) model. CHI was induced in adult CXCR2<sup>-/-</sup>, CXCR2<sup>+/-</sup> and wildtype (BALB/c) littermates by a weight-drop device. We found that neutrophil infiltration, assessed by NIMP-R14 immunohistochemistry on brain sections, was reduced by approximately 80% in CXCR2<sup>-/-</sup> mice at 12h and 7d post-CHI compared to wildtype (p<0.01; n=6/group). Whilst H&E-stained lesion volumes were similar at 12h, by 7 and 14d CXCR2<sup>-/-</sup> mice had significantly smaller lesions than wildtype mice (2-way ANOVA, p<0.001). This corresponded with a reduction in the density of TUNEL-labeled dead/dying cells in CXCR2<sup>-/-</sup> mice (p<0.05). Interestingly, marked elevation of the chemokines CXCL1 and CXCL2, as well as the growth factor G-CSF, was detected in CXCR2<sup>-/-</sup> brain homogenates by multiplex assay at 12 and 24h post-CHI (n=5; p<0.05). Functional recovery was assessed daily using a Neurological Severity Score (NSS) and a ledge beam test. Surprisingly, no improvement in neurological deficit was observed in CXCR2<sup>-/-</sup> mice compared to CXCR2<sup>+/-</sup> or wildtype mice. In conclusion, CXCR2 deficiency resulted in impairment of neutrophil infiltration into the injured brain, despite upregulation of chemokine levels. This corresponded with reduced neuronal loss and cell death following focal CHI. However, this did not correlate with any improvement in neurological outcome. Overall, this data supports a neurotoxic role for neutrophils in contributing to secondary brain damage following trauma.

## POS-MON-047

**ISCHAEMIA-INDUCED CAMKII PHOSPHORYLATION IN HYPERTENSIVE AND NORMOTENSIVE RATS**

Skelding K.A., Tomkins A., Fluechter L., Pepperall D., **Spratt N.** and Rostas J.A.P.

School of Biomedical Sciences and Pharmacy and Hunter Medical Research Institute, University of Newcastle, Callaghan, Australia.

Phosphorylation sites Thr286 and Thr253 are important regulators of CaMKII function. We examined the role of CaMKII phosphorylation in cell death/survival following experimental stroke. Middle cerebral arteries were occluded for 10, 15, 20, or 45 minutes (n=5/time-point) in normotensive Sprague-Dawley (SD) and spontaneously hypertensive rats (SHR). SHRs had substantially larger histological infarct volumes than SDs for each occlusion duration. We examined the role of CaMKII phosphorylation in cellular outcome following ischaemia. CaMKII phosphorylation at Thr286 (pThr286) or Thr253 (pThr253) in brain regions that are sensitive (striatum) or resistant (cortex) to ischaemic injury were measured at various times post-reperfusion (n=4-6/group). The post-reperfusion timecourse of pThr253 and pThr286 was different between normotensive and hypertensive rats. While a rapid rise of pThr253, but not in pThr286, was correlated with striatal cell death in SDs, there was no statistically significant change in either site in SHRs. We found that CaMKII levels in the striatum and cortex are equivalent in SHRs, whereas published data (Erondur, 1985) has shown significantly higher levels in cortex than in striatum in SDs. In addition to increased CaMKII expression, there appears to be an altered association between CaMKII and AMPA receptors in neurons in SHR resulting in enhanced CaMKII mediated excitotoxic cell death (Lecrux, 1997). In conclusion, we have demonstrated that vessel occlusions of short duration produce substantial infarction in SHRs compared with SD and phosphorylation patterns following ischaemia are different between strains. Although the use of SHRs reduces variability in stroke outcome, due to their genetically determined alterations in CaMKII expression/distribution, SHRs may not be a good model to examine CaMKII effects following stroke.

## POS-MON-048

**THE INVOLVEMENT OF NEUROTOXIN QUINOLINIC ACID IN NEUROPATHOGENESIS OF MULTIPLE SCLEROSIS**

**Lim C.K.<sup>1</sup>**, Adam S.<sup>1</sup>, Brew B.J.<sup>2,3</sup> and Guillemin G.J.<sup>1,2</sup>

<sup>1</sup>Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney NSW 2052, Australia. <sup>2</sup>St Vincent's Centre for Applied Medical Research, Sydney, Australia. <sup>3</sup>Department of Neurology, St Vincent's Hospital, Darlinghurst NSW 2010, Australia.

**Background:** The kynurenine pathway (KP) has increasingly drawn awareness in multiple sclerosis (MS), for which abnormal levels of KP metabolites have been found. Despite earlier studies showing that the KP may be activated and also the neuroprotective metabolite kynurenine acid production is increased. However, these data do not explain the detrimental effects of the KP in the neuropathology of MS. We hypothesize that this is associated with increased production of the excitotoxin quinolinic acid (QUIN). **Methods:** Our studies involve quantifying levels of tryptophan and several KP metabolites in the serum of patients with RRMS (n=51), SPMS (n=20) and PPMS (n=17) using HPLC and GC/MS. These patients had not received any recent corticosteroid treatment or other medications known to interfere with the KP at the time of sample collection. **Results:** We found that the kynurenine/tryptophan ratio in MS patients were significantly increased compared to control showing that the KP is activated at all the stages of MS. We also observed an increased production of QUIN in MS patients compared to controls. These data support the role of QUIN toxicity in MS pathogenesis.

## POS-MON-049

## TAM RECEPTOR SIGNALLING IN EARLY CNS DEMYELINATION

Ma G.Z.M.<sup>1</sup>, Kemper D.<sup>1</sup>, Kilpatrick T.J.<sup>1,2</sup> and Binder M.D.<sup>1,2</sup><sup>1</sup>Florey Neuroscience Institutes, University of Melbourne, Victoria 3010, Australia. <sup>2</sup>Centre for Neuroscience, University of Melbourne, Victoria 3010, Australia.

In the demyelinating disease multiple sclerosis, oligodendrocytes are the key cells damaged, with a subsequent loss of myelin. Microglia, the principal immune cells of the central nervous system, play important roles in the process of demyelination. Both oligodendrocytes and microglia express a family of protein tyrosine kinase receptors known as the TAMs (Tyro3, Axl and Mer), as well as their ligands Gas6 and Protein S (ProS). In this study, the early events of cuprizone-induced demyelination were examined in Gas6<sup>-/-</sup> mice. We found an increased loss of oligodendrocyte-lineage cells in Gas6<sup>-/-</sup> mice compared with Gas6<sup>+/+</sup> mice following 14 days of cuprizone challenge (1502±139.0 vs 864.7±118.7 cells/mm<sup>2</sup> for Gas6<sup>+/+</sup> and Gas6<sup>-/-</sup> mice respectively; p<0.001). Gas6<sup>-/-</sup> mice also displayed an increased oligodendrocyte precursor cell (OPC) response compared with Gas6<sup>+/+</sup> mice (88.8±6.2 vs 122.8±18.6 cells/mm<sup>2</sup> for Gas6<sup>+/+</sup> and Gas6<sup>-/-</sup> mice respectively; p=0.009). We have previously shown *in vitro* that Gas6 directly regulates oligodendrocyte survival and microglial activation [1]. In this study, we have found that ProS also promotes the survival of oligodendrocytes (35.2±1.2% vs 16.4±2.6% for 50nM Protein S and no factors respectively; p<0.001). However, unlike Gas6, ProS also promotes the proliferation of OPCs (15.1±1.8% vs 1.6±0.7% BrdU positive cells for 50pM ProS and no factors respectively; p<0.001). These data demonstrate the importance of TAM receptor signalling in promoting oligodendrocyte survival following a demyelinating insult, and emphasise the role of ProS as a ligand for the TAM receptors with potentially important effects that could enhance regeneration. 1. Binder et al (2008) J.Neurosci 8(20):5195-206.

## POS-MON-051

## MODULATION OF BONE MORPHOGENIC SIGNALLING DURING DEMYELINATION

Sabo J.<sup>1</sup>, Merlo D.<sup>1</sup>, Aumann T.<sup>1,2</sup>, Kilpatrick T.J.<sup>1,2</sup> and Cate H.<sup>1,2</sup><sup>1</sup>Florey Neuroscience Institutes. <sup>2</sup>Centre for Neuroscience, University of Melbourne.

Enhancement of endogenous oligodendrocyte regeneration is a promising strategy for repair in chronic demyelinating diseases of the central nervous system. Bone morphogenic proteins (BMPs) decrease the proliferation of neural precursor cells (NPCs) and inhibit the maturation of oligodendrocytes. Inhibiting BMP signalling during myelin injury could enhance oligodendrocyte production and remyelination. Here, we examine effects of modulating BMP signalling on oligodendrocyte precursor cells during demyelination. For *in vivo* studies, we have used the toxin-based model of demyelination, the cuprizone model, to induce central demyelination in the corpus callosum (CC). In the midline CC, BMP4 infusion increased p-SMAD 1/5/8 immunoreactivity 1.5-fold (p<0.05), whereas Noggin decreased p-SMAD 1/5/8 immunoreactivity (p<0.01). BMP4 infusion also resulted in significant increases in proliferation (p<0.01), Olig2+ cells (p<0.01), and Olig2+BrdU+ cells (p<0.01). Currently, we are examining effects of BMP4 and Noggin infusion after mice were allowed to recover by removing cuprizone from their diet for 1 week. Initial results show that Noggin infusion significantly increased the number of Olig2+ cells (p<0.05) and oligodendrocytes co-labelled with Olig2 and CC1 in the midline CC. In addition, Noggin infusion significantly increased proliferation (p<0.05), Olig2+BrdU+ cells (p<0.05) and CC1+BrdU+ cells (p<0.05). These findings demonstrate that BMP4 and Noggin infusion *in vivo* differentially regulated BMP signalling as indicated by p-SMAD 1/5/8 immunoreactivity. In the CC, the data suggests that BMP4 infusion is increasing the proliferation of oligodendrocyte precursor cells. After Noggin infusion and 1 week recovery, there was an increase in more mature oligodendrocytes in the CC. In the future, we will assess the effects of BMP4 and Noggin infusion on oligodendrocyte numbers and myelination following two week recovery from cuprizone-induced demyelination.

## POS-MON-050

## AN INDUCIBLE AND DEFINED DEMYELINATING CNS CO-CULTURE SYSTEM VISUALISED BY TIME-LAPSE CONFOCAL MICROSCOPY

Stratton J.A.S.<sup>1,2</sup>, Kilpatrick T.J.<sup>1,2</sup> and Merson T.D.<sup>1</sup><sup>1</sup>Florey Neuroscience Institutes. <sup>2</sup>Centre for Neuroscience.

Understanding the cellular and molecular responses to CNS demyelination could provide insight into pathogenic processes that occur at the earliest stages of lesion formation in multiple sclerosis (MS). Developing an *in vitro* model of CNS demyelination that enables real-time monitoring and manipulation of multiple independent variables in a fully defined culture system would offer many advantages. Here we describe progress towards generating such a model, comprising co-cultures of purified CNS retinal ganglion cells (RGCs) and oligodendrocyte progenitor cells (OPCs). OPCs were isolated from transgenic mice which express diphtheria toxin receptor (DTR) under the control of the oligodendrocyte-specific myelin basic protein (MBP) promoter. *In vitro* differentiation of MBP-DTR+ OPCs into mature myelinating oligodendrocytes results in the specific induction of DTR expression on mature oligodendrocytes rendering them sensitive to diphtheria toxin (DT)-mediated apoptosis. To date, we have established a rapidly myelinating co-culture system using mouse RGCs and OPCs (n=6). In addition, lenti-viral transduction of OPCs using a green fluorescent protein (GFP)-expressing virus prior to seeding onto dorsal root ganglion (DRG) neurons has enabled us to assess myelination in live cultures using time-lapse confocal microscopy (n=4). We have also demonstrated that the addition of 50 ng/ml DT to a RGC/ GFP+ OPC co-culture system results in the loss of oligodendrocytes only in MBP-DTR+ co-cultures in comparison to WT co-cultures (n=1). An *in vitro* model of CNS demyelination will provide a novel defined system to assess the role of specific factors and cell types implicated in the formation and evolution of early MS lesions and further our understanding of responses to degeneration of the axo-glial interface.

## POS-MON-052

## IDENTIFICATION OF POST-TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATORY MECHANISMS IN THE SYNAPTIC PROTEOME OF HUMAN, CIRRHOTIC-ALCOHOLIC BRAIN

Etheridge N.<sup>1</sup>, Mayfield R.D.<sup>2</sup>, Harris R.A.<sup>2</sup> and Dodd P.R.<sup>1</sup><sup>1</sup>SCMB, University of Queensland, St Lucia, QLD, Australia.<sup>2</sup>Waggoner Center for Alcohol and Addiction Research, University of Texas, Austin, Texas, USA.

Hepatic complications are a common side-effect of alcoholism. Without the detoxification capabilities of the liver, excess alcohol induces changes in protein expression throughout the body and brain. Proteomics was used to identify these protein changes in the brain. We utilised post-mortem human brain tissue from the superior frontal gyrus (SFG) of six cirrhotic-alcoholics, six uncomplicated alcoholics and six non-alcoholic and non-cirrhotic controls. Synaptic proteins were used in two-dimensional differential in-gel electrophoresis (2D-DIGE) coupled with mass spectrometry (MS). Many expression changes occurred only in either cirrhotic or non-cirrhotic alcoholics when compared to controls, suggesting that an alcoholic with cirrhotic complications may be responding to excessive drinking in a different manner to non-comorbid alcoholics. This was reiterated with the additional comparison of cirrhotic to non-cirrhotic alcoholics which showed that protein expression profiles within the SFG of these two alcoholic types were very different. There were many proteins identified in more than one spot on the 2D-gel indicating the presence of multiple protein isoforms caused by either post-transcriptional (i.e. splice variants) or post-translational regulation (i.e. protein modification). For some of these proteins, isoforms showed alcoholic-type-specific expression changes. For example, two isoforms of 70 kDa heat shock protein 1 were identified; one isoform was altered only in cirrhotic alcoholics when compared to controls, while the other was altered only in non-cirrhotic alcoholics. These types of proteins will be discussed in relation to post-transcriptional and post-translational regulatory mechanisms at work on proteins in the human alcoholic brain.



## POS-MON-053

## IMPACT OF ADVANCED PATERNAL AGE ON COPY NUMBER VARIATION IN OFFSPRING

Flatscher-Bader T.<sup>1</sup>, Foldi C.J.<sup>1</sup>, Chong S.<sup>2</sup>, Whitelaw E.<sup>2</sup>, Eyles D.W.<sup>1,3</sup>, Burne T.H.<sup>1,3</sup> and McGrath J.J.<sup>1,3</sup>

<sup>1</sup>Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia. <sup>2</sup>The Queensland Institute of Medical Research, Brisbane, QLD 4006, Australia. <sup>3</sup>Queensland Centre for Mental Health Research, Wacol, QLD 4076 Australia.

**Background:** Epidemiological studies revealed an association between paternal age and increased risk of autism spectrum disorders and schizophrenia in offspring. A role for *de novo* copy number variation (CNV) in these disorders has been suggested. Male germ line mutations accumulate with age and an increased CNV load may be propagated from older fathers to the offspring. We developed a mouse model to investigate CNVs in offspring of old fathers (advanced paternal age, APA) and of young fathers (young parental age, YPA). **Methods:** Young (3month) and old (14month) C57BJ/6J sires were mated to dams (3month) to generate 10 APA and 10 YPA offspring (5 males and 5 females within each group). Tail-tip DNA samples were hybridized competitively against a reference sample to 4x44k Agilent custom arrays containing probes targeting CNV regions, designed by S. Chong. CNVs were examined using the Agilent Genomic Workbench Suite, v5.0. The average number of CNVs between cohorts was compared with a Student's t-test. For 3 samples the assay was repeated with dye reversal. The initial and respective dye-swap experiments were combined for replicate analysis. **Results:** We found more deletions ( $P < 0.05$ ) in APA offspring compared to YPA offspring. Replicate analysis confirmed 55% of CNVs detected in the initial analysis. **Discussion:** These results provide preliminary evidence of an increased load of deletions in offspring of older fathers. Further validation and inclusion of parents in array-based screening will establish whether these changes will remain significant for *de novo* CNVs.

## POS-MON-055

## EFFECT OF INTRACISTERNAL ENZYME REPLACEMENT THERAPY ON CEREBROCORTICAL PATHOLOGY IN CANINE FUCOSIDOSIS

Kondagari G.S. and Taylor R.  
Faculty of Veterinary Science, University of Sydney, NSW, Australia.

**Introduction** Canine fucosidosis is an inherited lysosomal storage disorder caused by 14bp deletion leading to a deficiency of alpha-L-fucosidase. Vacuolation, perivascular storage, pyramidal neuronal loss, astrocytosis, myelin loss, microgliosis and axonal spheroid formation were observed at 2 months in early affected cortex prior to clinical signs of disease. Investigation of these markers of inflammation and degeneration is required to assess the impact of new therapeutic approaches such as direct enzyme replacement in fucosidosis. Therefore this study investigated pathological and molecular markers of early lysosomal storage in fucosidosis and determined the effects of repeated intracisternal enzyme replacement on these changes. **Methods** Animals were genotyped and grouped as affected enzyme treated (AET), affected vehicle treated (AVT) and control vehicle treated (CVT). They received enzyme or vehicle by intracisternal infusions monthly for 3 treatments. At necropsy cerebrocortical tissue was analysed for enzyme activity, substrates and gene expression and neuroinflammatory markers were quantified in immunostained cortical sections using image analysis. **Results** Increased enzyme activity correlated with decreased substrate storage ( $p < 0.05$ ). Significant decreases in LAMP1 gene expression and number of vacuoles/neuron were observed in AET compared with AVT ( $p < 0.05$ ). There were significant increases in IL6, IL8 and TGF $\beta$  gene expression in affected brain ( $p < 0.05$ ) and a trend to lower expression in treated versus control tissue. These findings correlated with reduced GFAP and lectin immunostaining in treated cortex. There was no difference in ubiquitin staining of axonal spheroids between AET and AVT. These tools provide sensitive markers of response to therapy for this disease. **Conclusions** Intracisternal enzyme infusions demonstrate potential as an early treatment and provide findings relevant to other neurodegenerative diseases.

## POS-MON-054

## GENE EXPRESSION PROFILING IN CANINE FUCOSIDOSIS

Fletcher J.L., Kondagari G.S., Williamson P. and Taylor R.  
The Faculty of Veterinary Science, The University of Sydney, NSW, Australia.

The deficiency of  $\alpha$ -L-fucosidase in the lysosomal storage disorder canine fucosidosis triggers a complex pathogenic cascade of neuronal dysfunction and death, neuroinflammation and myelin loss. This cascade begins early in disease and the molecular mechanisms that regulate it remain unclear. However, the neuroinflammatory element suggests that production of proinflammatory cytokines may have a key role. This study used microarray analysis to identify specific genes and pathways that contribute to the pathogenesis of canine fucosidosis. RNA from the cerebral cortex of fucosidosis affected ( $n=6$ ) and unaffected ( $n=3$ ) pups was hybridised to Affymetrix Canine Genome 2.0 GeneChips and gene expression intensities were analysed using R, BioConductor and Gene Set Enrichment Analysis (GSEA). GSEA allows microarray data to be interpreted based on the expression of functional gene groups, giving the analysis greater biological meaning. Selected results were confirmed with qRT-PCR. Significantly ( $P < 0.05$ ) up regulated genes included MHC II and lysosomal genes. GSEA also indicated significant ( $P < 0.01$ ; FDR  $< 0.05$ ) up regulation of lysosomal genes, consistent with the lysosomal enlargement seen in fucosidosis. Significantly ( $P < 0.05$ ) down regulated genes included FUCA1 and unexpectedly, several myelin structural genes. GSEA revealed that genes associated with oligodendrocyte differentiation and myelination were down regulated. This may be indicative of a dysregulatory mechanism causing myelin loss in this disease. GSEA of inflammatory and apoptotic pathways revealed up regulation of these pathways with indications that proinflammatory mediators such as tumour necrosis factor alpha may exacerbate neuronal loss via apoptosis. Gene expression profiling of canine fucosidosis using microarray analysis has provided fresh insight into the pathogenesis of fucosidosis and has provided new avenues of investigation and potential therapy development for this disease.

## POS-MON-056

## SELECTIVE ABLATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS DOES NOT AFFECT CONTEXTUAL FEAR CONDITIONING OR Y-MAZE PERFORMANCE

Hamlin A.S. and Coulson E.J.  
The Queensland Brain Institute, University of Queensland, Brisbane, Australia.

Neurodegeneration of basal forebrain cholinergic neurons (BFCNs) is an early and key feature of Alzheimer's disease. The BFCNs play a significant role in attention and contributes to learning and memory. It has been postulated that degeneration of these neurons contributes to the memory dysfunction associated with the onset of the Alzheimer's disease. To further elucidate the role of BFCNs in learning and memory we selectively ablated BFCNs and measured contextual fear conditioning and Y-maze performance. We hypothesised that ablation of BFCNs would lead to deficits in contextual learning and memory. Thirteen days prior to behavioural testing, male adult mice underwent stereotaxic surgery where the selective BFCN toxin murine-p75-saporin (0.4 $\mu$ g, 1 $\mu$ l) or the control toxin rabbit-IgG-saporin (0.4 $\mu$ g, 1 $\mu$ l) was infused bilaterally into the lateral ventricles. Choline acetyltransferase (ChAT) immunohistochemistry revealed a selective loss of BFCN cell bodies in the medial septum and vertical diagonal band of Broca and loss of BFCN terminal fields in the hippocampus and prefrontal cortex following intracerebroventricular infusion of murine-p75-saporin ( $p < 0.05$ ). Ablation of BFCNs had no effect on the level of freezing on test, following contextual fear conditioning ( $p > 0.05$ ). Furthermore, BFCN loss did not affect the amount of time mice spent in the novel arm in the Y-maze test ( $p > 0.05$ ). These data show that a significant reduction in BFCNs does not affect learning and memory when measured using contextual fear conditioning or Y-maze. Further investigation is required to determine the specific role that degeneration of BFCNs plays in memory dysfunction.



## POS-MON-057

## CORRELATIONS BETWEEN NEURONAL AND MICROGLIAL ACTIVATION INDUCED BY CHRONIC RESTRAINT STRESS IN THE RAT MEDIAL PREFRONTAL CORTEX

Hinwood M., Tyman R.J., Day T.A. and Walker F.R.  
School of Biomedical Sciences, The University of Newcastle,  
University Drive, Callaghan, NSW, 2308.

**Purpose:** Exposure to chronic stress induces proliferation of microglia, the main resident immunological cells of the CNS, in the rat brain. The activation of microglial cells by psychological stress may be due to neuronal signalling. In this study, we assessed the correlation between neuronal and microglial activation by chronic restraint stress in the medial prefrontal cortex, an area of the brain particularly responsive to stress. **Methods:** Rats received 21 daily exposures to either six hours of restraint (stress; n=9), or to twice-daily handling with six hours of food and water deprivation (handled control; n=8). Following stress, animals underwent sucrose preference testing. On day 22, 24h after the final exposure to stress or handling, rats were perfused transcardially with sodium nitrite and paraformaldehyde (4%), then immunohistochemistry for both  $\Delta$ FosB, a marker of chronically activated neurons, and microglial marker IBA-1, was performed in consecutive sections. **Results:** We observed a decrease in sucrose preference and a reduction in weight gain in animals exposed to stress, but not in handled controls. Exposure to restraint stress significantly elevated numbers of both  $\Delta$ FosB-positive cells and IBA-1-positive cells, in the infralimbic medial prefrontal cortex ( $p < .05$ ). Additionally, numbers of  $\Delta$ FosB-positive cells were positively correlated with numbers of microglia ( $r = 0.64$ ). **Conclusion:** These results demonstrate that numbers of stress-responsive neurons and microglial cell counts are positively correlated. Neurons expressing  $\Delta$ FosB after exposure to chronic stress may play a role in triggering stress-induced microglial activation and neuroinflammation in the medial prefrontal cortex.

## POS-MON-059

## EXPRESSION OF ABCA8 IN HUMAN BRAIN WHITE MATTER: POTENTIAL ROLE IN SPHINGOMYELIN HOMEOSTASIS

Kim W.S.<sup>1,2</sup>, Bhatia S.<sup>1</sup>, Shannon Weickert C.<sup>1,2</sup>, Halliday G.M.<sup>1,2</sup> and Garner B.<sup>1,2</sup>

<sup>1</sup>Prince of Wales Medical Research Institute, Randwick NSW 2031, Australia. <sup>2</sup>School of Medical Sciences, University of New South Wales, Sydney NSW 2052, Australia.

ABCA8 is a recently discovered ATP-binding cassette (ABC) transporter. Specific ABC transporters contribute to lipid transport in the central nervous system, however, very little is known regarding ABCA8 function in the human brain. In the present study we used a combination of Affymetrix microarray gene analysis and quantitative real-time PCR to conduct a comprehensive mapping of this gene in 13 regions of normal adult human brain (n=6) and in the developing human prefrontal cortex ranging in age from 39 days to 49 years (n=45). In the prefrontal cortex cohort, the expression of ABCA8 increased with age with a sharp increase during the toddler years (1.58 to 4.86 years). In the adult human brains, ABCA8 was differentially expressed in all 13 regions examined with particularly high expression detected in superior frontal white matter and inferior temporal white matter. Since ABCA8 was highly expressed in white matter, we investigated a potential relationship between ABCA8 and factors that control the regulation of the major white matter lipid, sphingomyelin, in vitro. Transfection of human MO3.13 oligodendrocytes with ABCA8 significantly increased the expression of sphingomyelin synthase 1 mRNA by 2.4 fold ( $p=0.005$ , n=2 experiments). Interestingly, when oligodendrocytes were treated with the sphingomyelin synthesis inhibitors D609 (0.2 mM) or myricocin (0.1 mM) for 24 h, the expression of ABCA8 was significantly increased by 4.6 fold ( $p=0.005$ ) and 2.9 fold ( $p=0.05$ ) respectively (n=2 experiments). These data indicate a novel relationship between ABCA8 and sphingomyelin homeostasis that warrants further detailed investigation.

## POS-MON-058

## LONG-TERM CHANGES IN NEUROPROTEIN EXPRESSION IN VOLUNTARILY MORPHINE PREFERRING RATS

Joshi D., Malone D.T. and Taylor D.A.  
Monash Institute of Pharmaceutical Sciences, Monash University, 381  
Royal Parade, Parkville 3052, Victoria, Australia.

Individual vulnerability to develop preference to drugs of abuse remains a major challenge in addiction research in that long-lasting neuroprotein alterations underlying addictive behaviour are still poorly understood. Using a voluntary oral morphine self-administration model, we have investigated the long-term neuroprotein [opioid receptors ( $\mu$  {MOR} and  $\delta$  {DOR}), dopamine receptors ( $D_2$  and  $D_3$ ), the cannabinoid CB<sub>1</sub> receptor, synaptic plasticity markers (synapsin I and synaptophysin) and phosphorylated-cAMP response element binding protein (pCREB)] differences in reward-specific brain regions of rats showing preference to self-administer morphine (HMP) compared to low/non-morphine preferring rats (LMP). 40 male Sprague Dawley rats were exposed to increasing concentrations of morphine in their sucrose-flavoured drinking water for 3 weeks. Following one week drug free period, rats were given a 3 week choice between a morphine containing sucrose solution and a sucrose solution only. Based upon their morphine intake they were classified as HMP or LMP. A week later, half the rats from each group (HMP and LMP) were given a 3 week second choice phase (CP2 - voluntary) or a 3 week second no choice phase (NCP2 - involuntary). A week later, rat brains were fixed for immunohistochemical analysis. Only the HMP rats which voluntarily self-administered morphine (HMP.CP2) had a significant increase in  $D_2$  receptor,  $D_3$  receptor, MOR, synapsin I, synaptophysin and pCREB expression in reward-specific brain regions. In conclusion, the results suggest that the observed changes in neuroprotein expression levels of HMP.CP2 are causal of or a consequence of morphine preference and are not due to involuntary high morphine intake (HMP.NCP2) over the same time period. These changes appear to be related to synaptic plasticity in reward-specific brain regions of voluntarily morphine preferring rats.

## POS-MON-060

## PATIENT-DERIVED, HUMAN ADULT OLFACTORY STEM CELLS: A NEW MODEL FOR NEUROLOGICAL DISEASES

Mackay-Sim A.<sup>1</sup>, Abrahamsen G.<sup>1</sup>, Matigian N.<sup>1</sup>, Sutharsan R.<sup>1</sup>, Nouwens A.<sup>2</sup>, Cochrane J.<sup>1</sup>, Perry C.<sup>1</sup>, Silburn P.<sup>1</sup>, McGrath J.<sup>1</sup> and Wells C.<sup>1</sup>

<sup>1</sup>National Centre for Adult Stem Cell Research, Griffith University.

<sup>2</sup>Australian Institute of Biotechnology and Nanotechnology, University of Queensland.

Most brain diseases are caused by multiple genes of small effect interacting with environmental risk factors and a major goal of stem cell research is to develop cellular models of neurological disease. The olfactory epithelium is an accessible neural tissue that regenerates throughout adult human life and contains a multipotent stem cell. Previously we showed increased cell proliferation and altered adhesion in olfactory biopsies from patients with schizophrenia compared to healthy controls. Here we tested the hypothesis that olfactory stem cells from the nose of patients provide a model for brain conditions and diseases. We generated olfactory stem cell lines, grown as neurospheres, from 42 people: 9 with schizophrenia, 19 with Parkinson's disease, and 14 healthy controls. Gene expression profiling was undertaken using the Illumina Human Ref 8v2 BeadArray. 1700 and 514 transcripts were differentially expressed exclusively in schizophrenia and Parkinson's disease stem cells, respectively. Protein expression profiling was undertaken using 2D-DIGE in a subset of 9 cell lines (3 each from patients and controls) and Western blots on another subset of cell lines. Cell functions were assessed with a battery of multi-well plate assays. Pathway analysis revealed gene and protein networks and cell signalling pathways altered in disease stem cells. We demonstrated significant disease-specific alterations in gene expression, protein expression and cell function including dysregulated neurodevelopmental pathways in schizophrenia and dysregulated mitochondrial function, oxidative stress and xenobiotic metabolism in Parkinson's disease. The cells revealed new candidate genes and cell pathways for future investigation. Fibroblasts from SZ patients did not show these differences. Olfactory stem/progenitor cultures provide an alternative to iPS and ES cells as disease models. They do not require genetic re-programming and they can be obtained from adults with complex genetic diseases. They will be useful for understanding aetiology, for diagnostics and for drug discovery.

## POS-MON-061

## PITUITARY VOLUME IN FOCAL EPILEPSY

Miazoi Z.<sup>1,2</sup>, Salzberg M.<sup>1</sup>, Lorenzetti V.<sup>1</sup>, O'Brien T.J.<sup>1</sup>, Velakoulis D.<sup>1,2</sup> and Adams S.<sup>1,2</sup>

<sup>1</sup>University of Melbourne, Parkville. <sup>2</sup>Melbourne Neuropsychiatry Centre, St Albans.

**Background:** The hypothalamic-pituitary-adrenal (HPA) axis is important in mesial temporal lobe epilepsy (MTLE). Medial temporal lobe pathology or seizures and/or comorbid depression may lead to HPA axis hyperactivity which may in turn lead to anterior pituitary enlargement. We hypothesised that patients with MTLE, particularly those with a history of depression, would exhibit enlarged pituitary gland volumes (PGV).

**Methods:** We investigated 81 patients with medically refractory focal epilepsy and 73 healthy controls. DSM-IV psychiatric diagnoses were obtained from prior psychiatric interview. PGV and intracranial volume (ICV) were measured using a manual region of interest methodology on volumetrically acquired 1.5mm thick coronal T1-weighted MR images.

**Results:** There was no relationship between ICV and PGV. Patients with MTLE had 14% smaller PGV compared to controls, and 13% smaller PGV compared to patients with extratemporal lobe epilepsy (ETLE). Patients with a history of depression (n=20) had significantly larger PGV than those without. MTLE patients had 8% smaller ICV than ETLE patients, and 6% smaller ICV than controls. **Conclusions:** Contrary to our hypothesis we identified smaller PGV in patients with MTLE compared to control subjects and patients with ETLE. PGV and ICV reductions in MTLE patients differentiate this form of focal epilepsy from other focal epilepsies and suggest a neurodevelopmental basis for MTLE. Chronic excessive glucocorticoid exposure inhibits the activity of other anterior pituitary hormones, which may mask enlargement of corticotrophs, accounting for small PGV. Enlarged pituitary volumes in those with a history of depression suggests a dysfunctional HPA axis may be associated with pathophysiology of depressive disorders in patients with focal epilepsy. **Correspondence:** Sophia Adams, Royal Melbourne Hospital, Level 2, John Cade Building, Melbourne Sophia. Adams@mh.org.au.

## POS-MON-062

## OLANZAPINE DECREASES [3H]SR141716A BINDING TO CANNABINOID CB1 RECEPTORS IN THE RAT HIPPOCAMPUS AND AUDITORY CORTEX: A DOSAGE-DEPENDENT RESPONSE

Lian J.<sup>1</sup>, Weston-Green K.<sup>1,2</sup>, Kang K.<sup>1,3</sup>, Huang X.-F.<sup>1,2</sup> and Deng C.<sup>1,2</sup>

<sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, NSW 2522, Australia. <sup>2</sup>Schizophrenia Research Institute, NSW 2010, Australia. <sup>3</sup>Ninth Hospital of Chongqing, Chongqing 400700, China.

Olanzapine is widely used in clinics due to its efficacy and tolerability in the treatment of multiple domains of schizophrenia. Abnormalities of the endocannabinoid system have been found in schizophrenia. The hippocampus is implicated in the pathophysiology of schizophrenia and the auditory cortex (AC) plays a role in auditory hallucinations. This study investigated the effects of olanzapine on cannabinoid CB1 receptors (CB1R) in the hippocampus and AC. **Methods:** Female Sprague Dawley rats (n=6/group) were administered with olanzapine (0.75, 1.5, 3.0, 6.0mg/kg/day, 3x/day, orally) or vehicle (control) for 2-weeks. The density of [3H]-SR141716A (a selective antagonist) binding site to CB1 receptors in the hippocampus and AC were captured using a Beta-Imager. **Results:** For the AC, 3mg/kg/day and 6mg/kg/day olanzapine treatments significant decreased CB1R binding densities compared to the control group (p<0.05). Olanzapine at 1.5mg/kg/day tended to decrease CB1R binding (p=0.067) in the auditory cortex. For the hippocampus, ANOVA showed a significant effect of olanzapine (p<0.05). Further post-hoc analyses showed that the doses of 1.5, 3 and 6mg/kg/day tended to decrease CB1R binding in the hippocampus. However, very low dose (0.75mg/kg/day) olanzapine had no effect on CB1R binding in both hippocampus and AC. **Conclusion:** These results suggest the existence of dose-dependent response between olanzapine treatment and CB1R binding sites in the hippocampus and auditory cortex. These changes may contribute to olanzapine efficacy in ameliorating schizophrenia symptoms via CB1R, at least at higher doses.

## POS-MON-063

## THE DOSAGE RESPONSE TO THE SIDE-EFFECT OF METABOLIC DYSFUNCTION IN FEMALE RATS FOLLOWING OLANZAPINE TREATMENT

Weston-Green K.L.<sup>1,2</sup>, Huang X.F.<sup>1,2</sup> and Deng C.<sup>1,2</sup>

<sup>1</sup>Centre for Translational Neuroscience, University of Wollongong. <sup>2</sup>Schizophrenia Research Institute.

Olanzapine is commonly prescribed to treat schizophrenia, but can induce metabolic dysfunction by largely unknown mechanisms. Clinical reports suggest olanzapine alters satiety signals and behavioural activity, however findings appear conflicting. Previous animal model studies have utilised a range of olanzapine dosages, however the dosage that better mimics the human scenario of olanzapine-induced weight gain is unclear. **METHODS:** Female Sprague Dawley rats were treated with olanzapine (0.75, 1.5, 3.0, 6.0mg/kg/day, orally 3x/day) or vehicle (control) (n=12/group) for 14 days. Body weight, food and water intake were recorded. Behaviour was examined using open field (OFT) and elevated plus maze (EPMT) testing. The concentrations of plasma leptin, insulin, ghrelin and glucose were measured. Subcutaneous and intra-abdominal white fat and inter-scapular brown adipose tissue (BAT) were weighed. **RESULTS:** Olanzapine increased body weight (1.5-6.0mg), food intake (6.0mg) and feeding efficiency (1.5-6.0mg), with no effect on water intake. Subcutaneous inguinal (3.0-6.0mg) and intra-abdominal perirenal fat was increased (6.0mg), but not BAT. Low doses of olanzapine (0.75-1.5mg) induced hypoleptinemia, whilst high doses (3.0-6.0mg) increased leptin. Ghrelin increased in all dosage groups. Olanzapine decreased insulin (0.75-6.0mg), glucose (1.5-6.0mg), and locomotion in OFT (1.5-6.0mg), with no change in EPMT. 0.75mg/kg/day had no effect on most parameters measured. **CONCLUSION:** Olanzapine-induced weight gain is associated with hyperphagia, enhanced feeding efficiency and adiposity, decreased locomotion and altered satiety signaling. The animal model used in the present study (oral olanzapine at dosage range: 1.5-6.0mg/kg/day, but not 0.75mg/kg/day) mimics aspects of the clinic, with a dosage-response evident in most parameters measured and a maximal effect following 6.0mg/kg/day olanzapine.

## POS-MON-064

## SMALL ANIMAL PET WITH [18F]FDG AFTER A SINGLE DOSE OF THE SYNTHETIC CANNABINOID HU210

Nguyen V.H.<sup>1</sup>, Wang H.<sup>1</sup>, Verduran M.<sup>1,2</sup>, Dedeurwaerdere S.<sup>1</sup>, Zahra D.<sup>1</sup>, Gregoire M.-C.<sup>1</sup> and Zavitsanou K.<sup>1,2</sup>

<sup>1</sup>Radiopharmaceuticals Research Institute, ANSTO, Menai NSW 2234, Australia. <sup>2</sup>Schizophrenia Research Institute, Sydney NSW 2000, Australia.

Cannabis use has been shown to alter brain metabolism in both humans and animal models. [<sup>18</sup>F]-2-fluoro-2-deoxy-D-glucose (FDG) is a glucose analogue used as a tracer of glucose metabolism. In the brain, FDG becomes trapped in cells after phosphorylation by hexokinase, in a distribution reflective of metabolic neuronal activity. **Aim:** To investigate the effects of a single-dose injection of the synthetic cannabinoid HU210 on glucose metabolism in the rat brain using [<sup>18</sup>F]FDG small animal PET. **Methods:** Adult male Wistar rats (70-77 days old) were received a single-dose of the synthetic cannabinoid HU210 (100 µg/kg, n=7) or vehicle (n=5). Approximately 1 mCi of [<sup>18</sup>F]FDG was i.v. injected into each animal at 15-min and 24-hr post-injection of HU210 (Day 1 & 2, respectively). A 20-min PET scan was performed at 40-min after each [<sup>18</sup>F]FDG injection. Standardised Uptake Values (SUVs) were calculated from 22 brain regions for each animal. **Results:** (1) Overall increased SUVs in whole brains, hence glucose utilisation, were observed in the treatment group compared to the vehicle-treated controls on day 1 (14%, p<0.0001), but not on day 2. However, no significant difference in SUVs between individual brain regions was observed between HU210 and vehicle-treated rats; (2) In the control group, no changes were observed in SUVs on both day 1 and day 2. In the treatment group, however, overall SUVs were decreased by 19% on day 2 from that of day 1 (p<0.0001). **Conclusion:** A single high dose of HU210 increased glucose utilisation in brain cells. This result mirrors human studies showing increased brain activation after acute administration of cannabinoids.

## POS-MON-065

**AN ABSTINENCE MODEL OF CUE-INDUCED REWARD-SEEKING REVEALS SIMILAR RELAPSE BEHAVIOUR FOR BOTH NATURAL AND DRUG REINFORCERS IN MICE**Madsen H.B.<sup>1,2</sup>, Brown R.M.<sup>1,3</sup> and Lawrence A.J.<sup>1,2</sup><sup>1</sup>Florey Neuroscience Institutes, Parkville, Vic, 3010. <sup>2</sup>Centre for Neuroscience, University of Melbourne, Parkville, Vic, 3010. <sup>3</sup>Monash Institute of Pharmaceutical Sciences, Parkville, Vic, 3052.

Addiction is a complex disease which is characterised by a high propensity to relapse despite prolonged abstinence. Relapse behaviour can be assessed in animals using models of drug-seeking, however the critical brain structures underlying such behaviours are yet to be explored in mice. This study compared cue-induced reward-seeking of mice trained to respond for either morphine or sucrose, and subsequently examined the regional expression of Fos, a marker of neuronal activation. Adult male mice on a CD1 background were placed in operant chambers equipped with both active and inactive levers, and trained to lever press in order to obtain either a sucrose reward (10% w/v) or intravenous morphine (0.1mg/kg/infusion). Once stable operant responding was established, mice were subjected to withdrawal in their homecages for 3 weeks and then returned to the operant chambers to assess reward-seeking behaviour in the presence of drug-associated cues, or killed for tissue collection. Immunohistochemistry was used to examine the expression of Fos in sucrose relapse (n = 4), morphine relapse (n=5), sucrose withdrawal (n=5), morphine withdrawal (n=5) and naïve (n=5) mice. Mice trained to self-administer both sucrose and morphine exhibited robust responding on the active lever on relapse day when compared to the inactive lever (p<0.05). There was no difference between the two groups of mice indicating similar reward-seeking behaviour in response to both drug and natural reward. Analysis of Fos expression revealed significant activation of the lateral hypothalamus for both the sucrose and morphine relapse groups (p<0.05) implicating this brain region in reward-seeking behaviour.

## POS-MON-067

**THE EFFECTS OF CHRONIC ADOLESCENT AND ADULT AMPHETAMINE TREATMENT ON PSYCHOTIC-LIKE BEHAVIOURS AND SYNAPTIC PLASTICITY**Malone D.T., Cassells K., Short J.L. and Taylor D.A.  
Monash Institute of Pharmaceutical Sciences, Monash University.

Schizophrenia is a debilitating neuropsychiatric disorder which is characterised by a number of symptoms. These include positive symptoms (hallucinations, delusions), negative symptoms (social withdrawal, emotional flatness) and memory deficits. It has been postulated that the onset of these symptoms is the result of a disruption in neuronal plasticity during early development. Recent research has demonstrated that withdrawal from an administration of an escalating-dose of amphetamine induces behavioural sensitisation in rodents which closely resembles positive symptoms of schizophrenia. The present study employed an escalating amphetamine administration schedule to determine an animal model which represents the three symptoms associated with schizophrenia. Male Sprague-Dawley rats (n = 12 per group) were given a dosage ranging from 1 to 12 mg/kg during adulthood (pnd 55 to 60) or adolescence (pnd 34 to 39). After a period of withdrawal, behavioural paradigms relevant to schizophrenia were used to determine the presence of deficits in pre-pulse inhibition (PPI), social interaction and spatial recognition memory in the adult brain. It was demonstrated that PPI was disrupted in subjects treated during adolescence, however this was only apparent after 3 weeks of withdrawal and was not detected in adult-treated subjects. No deficits in social interaction or spatial recognition memory were established for any treatment group. In addition, immunohistochemistry assays conducted determined significant reductions in the synaptic proteins synapsin and synaptophysin in the striatum and nucleus accumbens in adolescent amphetamine treated rats. These results suggest that the amphetamine administration schedule adopted in the present study produced some psychotic-like deficits, but was unable to produce a full range of behaviours similar to that observed in schizophrenia.

## POS-MON-066

**PLASMA LEPTIN AND SALIVARY CORTISOL LEVELS ARE CORRELATED WITH STATE ALCOHOL AND SMOKING CRAVING IN EARLY ABSTINENT ALCOHOLICS**Ho A.M.C.<sup>1</sup>, Daglish M.R.<sup>1</sup>, Dodd P.R.<sup>2</sup> and Stadlin A.<sup>3</sup><sup>1</sup>Discipline of Psychiatry, School of Medicine, University of Queensland, Brisbane, Australia. <sup>2</sup>School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia. <sup>3</sup>Department of Anatomy, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea.

Appetite-regulating and stress hormones have received much attention in alcohol research in recent years, due to their potential involvement in the regulation of alcohol consumption patterns and craving level. The relationship between craving states for alcohol, smoking, food and water with leptin levels have not been explored. We examined the associations among these craving states, and with cortisol and leptin concentrations, in early-abstinent alcoholics. Alcohol-dependent subjects (56 males, 40 females) were recruited during detoxification. On day 4 of withdrawal, subjects were instructed to fast overnight. A self-report state measure, Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995) was used to measure alcohol craving the next morning before breakfast. Craving scores for smoking (smokers only), food and water were concurrently assessed using an AUQ-derived questionnaire. Saliva and fasting blood samples were collected immediately afterwards. Cortisol and leptin levels were determined by ELISA. Results showed that state alcohol craving was significantly correlated with smoking and water craving levels (r = 0.319, P = 0.006 and r = -0.230, P = 0.024 respectively). A significant correlation was found between levels of craving for food and water (r = 0.257; P = 0.012). In female alcoholics, salivary cortisol and BMI-corrected plasma leptin both showed positive linear correlation with alcohol craving (P = 0.001 and P = 0.075 respectively). In male alcoholics, salivary cortisol level correlated only with smoking craving (r = 0.407, P = 0.054). These preliminary results suggest that state alcohol craving may be correlated with state cravings for smoking and water. Gender-specific correlations may be present between levels of plasma leptin and salivary cortisol as well as state cravings for alcohol and smoking during early withdrawal.

## POS-MON-068

**CALORIC VESTIBULAR STIMULATION REDUCES ALLODYNIA IN COMPLEX REGIONAL PAIN SYNDROME (CRPS) TYPE II**Ngo T.T.<sup>1,3</sup>, Chou M.J.<sup>4</sup>, Nunn A.<sup>2,4,5</sup>, Arnold C.<sup>3</sup>, Brown D.J.<sup>5</sup>, Gibson S.J.<sup>3,6</sup> and Miller S.M.<sup>1,3,5</sup><sup>1</sup>Perceptual and Clinical Neuroscience Group, SPPPM, and <sup>2</sup>Dept ECSE, Monash University, Melbourne, Australia. <sup>3</sup>CPMRC and <sup>4</sup>Amputee Unit, Caulfield Hospital, Melbourne, Australia. <sup>5</sup>VSCS, Austin Health, Melbourne, Australia. <sup>6</sup>NARI, University of Melbourne, Melbourne, Australia.

Caloric vestibular stimulation (CVS) is a common vestibular diagnostic test that induces a range of remarkable phenomenological effects, including pain reduction following limb deafferentation, spinal cord injury (SCI) and stroke (Miller & Ngo, 2007, *Acta Neuropsychiatr* 19:183-203). CVS also reportedly decreases post-stroke allodynia (McGeoch et al., 2009, *Acta Neurol Scand* 119:404-409). We report a case study of a 54 year-old woman with CRPS II (Budapest Criteria) following wrist fracture with median nerve injury. The patient reported low baseline pain levels (1.5/10 on a visual analogue scale) but for several weeks had marked allodynia to light touch (6-8/10). Iced-water CVS was administered on three consecutive days. On day 1 CVS caused a small pain reduction but allodynia decreased substantially from 8/10 to 4/10. By day 2 allodynia had further decreased to 1/10 (with no further change from the second CVS). Allodynia was 2/10 by day 3, decreasing to 1/10 after a third CVS administration. Allodynia remained low (1/10) at day 6 and the patient remarked being able to wear long-sleeve garments for the first time since developing CRPS II. Allodynia began increasing at day 8, and 1 week later the patient requested further CVS administration to help manage it. We are investigating the use of repeated CVS as a potential pain management tool in CRPS, phantom limb pain and SCI pain. Further preliminary findings will be presented, and the neurobiology of CVS and its pain-reduction effects will be discussed.



## POS-MON-069

**SPECIFIC, NON-VIRAL GENE DELIVERY TARGETING MOTOR NEURONS IN-VITRO AND IN-VIVO**

**Rogers M.-L.<sup>1</sup>**, Matusica D.<sup>1</sup>, Hoffman L.<sup>2</sup>, Voelcker N.H.<sup>2</sup> and Rush R.A.<sup>1</sup>  
<sup>1</sup>Department of Human Physiology, School of Medicine, Flinders University, GPO Box 2100 Adelaide 5001. <sup>2</sup>School of Chemistry, Physics and Earth Sciences, Flinders University, GPO Box 2100 Adelaide 5001.

**Purpose:** Receptor specific, non-viral gene delivery vehicles provide a way to deliver therapeutic agents for motor neuron disease. We have constructed a highly specific non-viral gene delivery agent targeting the common neurotrophin receptor (p75NTR) in-vitro and in the SOD1<sup>G93A</sup> transgenic mouse model of motor neuron disease in-vivo. **Methods:** Polyethylene glycol (PEG) was conjugated to polyethylenimine (PEI) and characterised by NMR. The PEI-PEG construct was then conjugated to a monoclonal antibody to p75NTR (clone MLR2) and assessed for ability to condense GFP plasmid DNA (pGFP) electrostatically. The size, zeta potential and DNase protection ability of MLR2-PEI-PEG-pGFP was compared to PEI-PEG-pGFP. MLR2-PEI-PEG-pGFP was then tested for ability to target primary motor neurons from embryonic SOD1<sup>G93A</sup> mice and adult SOD1<sup>G93A</sup> transgenic mice. **Results:** PEI was effectively PEGylated as determined by NMR. The MLR2-PEI-PEG construct condensed and bound pGFP at a nitrogen to phosphate ratio (N/P) of 3.5 to 10. The resulting DNA nanoparticles (MLR2-PEI-PEG-pGFP) had a negative zeta potential and a size of less than 100 nm (n=3). PEGylated PEI-MLR2 protected pGFP from DNase digestion (n=3). Importantly, MLR2-PEI-PEG was able to specifically target motor neurons in mixed cultures containing embryonic primary motor neurons and glia from SOD1<sup>G93A</sup> mice (n=3). Finally, MLR2-PEI-PEG-pGFP delivered into adult SOD1<sup>G93A</sup> mice by intraperitoneal injections, resulted in GFP expression in spinal motor neurons (n=4). **Conclusions:** This study shows effective non-viral gene delivery to motor neurons in vitro and in vivo. Further work is ongoing to demonstrate that this agent delivers therapeutic genes to SOD1<sup>G93A</sup> mice.

## POS-MON-070

**CHRONIC STRESS SUFFICIENT TO ELICIT ANHEDONIA ALTERS THE DENSITY AND MORPHOLOGY OF MICROGLIA IN FOREBRAIN AND MIDBRAIN STRESS RESPONSIVE NUCLEI**

**Tynan R.J.<sup>1,3</sup>**, Naicker S.<sup>1,3</sup>, Hinwood M.<sup>1,3</sup>, Nalivaiko E.<sup>1,3</sup>, Buller K.<sup>2</sup>, Day T.<sup>1,3</sup> and Walker R.<sup>1,3</sup>

<sup>1</sup>School of Biomedical Science and Pharmacy. <sup>2</sup>University of Queensland. <sup>3</sup>Hunter Medical Research Institute. <sup>4</sup>University of Newcastle.

The aim of the current study was to evaluate changes in microglial activation status in stress responsive forebrain and midbrain nuclei following exposure to chronic restraint stress. The study consisted of two separate experiments with each experiment having identical stress and control groups. Our stress protocol involved 2 x 30 min of randomly administered restraint sessions per day for 14 consecutive days. In the first experiment, we evaluated a variety of behavioural and physiological parameters including sucrose preference, weight gain, core body temperature and behavioural adaptation to stress exposure. In the second experiment, we investigated using immunohistochemistry a variety of microglial activation markers including ionized calcium binding adaptor molecule-1 (Iba-1) and major histocompatibility complex II (MHC-II) in a total of 14 stress responsive nuclei. Additionally, we investigated cellular proliferation using Ki67 labelling in the same anatomical regions. The results from the study demonstrate that chronic stress induced a significant increase in anhedonia (p<.05), a decrease in weight gain across the entire observation period (p<.05), a significant elevation in core body temperature during restraint (p<.05) and a progressive decrease in struggling behaviour (p<.05). In regard to microglial activation, it was apparent that chronic stress induced a significant upregulation in the density of Iba-1 labelling (8 of 14 regions) and number (7 of 14 regions) of Iba-1 positive cells (all p's <.05). Within the regions that exhibited an increased number of Iba-1 positive cells following chronic stress, we found no evidence of a between group difference in MHC-II labelling. However, we did find evidence of an increase in Ki67 positive cells within the dentate gyrus. In summary, these results clearly demonstrate that chronic stress increases the number of microglia, and further causes a marked transition of microglia from a ramified-resting state to a hypertrophic-activated state.

## POS-MON-071

**A TURNKEY TUTORIAL WITH THE SIMULATOR SIMBRAIN 3 TEACHES STUDENTS ABOUT CONNECTIONIST NEURAL NETWORKS**

**Holcombe A.O.<sup>1</sup>** and Yoshimi J.<sup>2</sup>

<sup>1</sup>School of Psychology, University of Sydney. <sup>2</sup>School of Social Sciences, Humanities, and Arts, University of California Merced.

Many neuroscience students graduate without a firm understanding of how networks of connected neurons mediate adaptive behaviour. Our 90-minute tutorial ([www.psych.usyd.edu.au/staff/alexh/teaching/neuralNets/](http://www.psych.usyd.edu.au/staff/alexh/teaching/neuralNets/)) provides undergraduates with an interactive, engaging experience that facilitates such an understanding. The connectionist simulator Simbrain (Yoshimi, 2008) is Java-based software for building and analyzing neural networks using an intuitive graphical interface (neurons can be selected and dragged by the mouse, copied and pasted, edited with a double-click, etc). First, step-by-step instructions lead students through an exercise with a two-layer, eight-neuron network that controls a virtual mouse. Simple abstracted olfactory neurons are connected to motor neurons so that the mouse is guided toward a piece of cheese. Students add connections to the network to make the mouse also approach another object. Next, students attempt to re-wire the network so that the mouse does not approach the objects if they are in the same location (which illustrates the concept of exclusive-or). Many arrive at a solution themselves, and the subsequent explanation further communicates the power of simple networks. In a second set of exercises, students begin with a fully connected network of neurons with connections that change according to the Hebb rule. Students teach the network a pattern, provide a partial cue, and watch pattern completion unfold. After training on more patterns students see interference between non-orthogonal patterns and more fully appreciate how a connectionist memory works. In summary, students see neural networks come to life with these simulations. By facilitating exploration, tinkering, and active discovery, our experience suggests these tutorials promote understanding more effectively than a lecture.

## POS-MON-072

**ONLINE FEEDBACK ASSESSMENTS IN PHYSIOLOGY: EFFECTS ON LEARNING**

**Ulman L.G.<sup>1</sup>**, Marden N.Y.<sup>1</sup> and Velan G.M.<sup>2</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences, The University of New South Wales, Sydney 2052 Australia. <sup>2</sup>Department of Pathology, School of Medical Sciences, The University of New South Wales, Sydney 2052 Australia.

Online formative assessments are an increasingly popular supplement to traditional summative exams in higher education, however formal evidence supporting their educational benefits is lacking. This study evaluated the impact of online feedback quizzes on the learning outcomes of the cohort of science students enrolled in our stage 1 undergraduate Physiology course in session 1 2009. Three online feedback quizzes were offered during the 12 week course. To encourage student participation and preparation, each quiz was worth 5% of the overall course credit. Quizzes consisted of 10 multiple choice questions on a specific section of the course material. Summative end of session examination marks were analysed with respect to performance in quizzes, and were also compared to those achieved by students completing the course in session 1 2008, in which the quizzes were not offered. A survey was conducted to gather students' perceptions regarding the quizzes. There were no significant differences in the end of session examination marks between the 2008 and 2009 student cohorts. However, there was a significant relationship between performance in the quizzes and performance in the end of session examination ( $r^2=0.231$ ,  $n=450$ ,  $P<0.001$ ). Further, students who performed poorly in the quizzes were more likely to perform poorly in the end of course examination. Survey results were generally favourable, with the majority of students identifying the quizzes as a valuable learning tool. These findings suggest that the online quizzes are good predictors of final exam performance and can be utilised to target students in need of remediation and assistance.



## POS-MON-073

**EXAMINING EXPRESSION OF HCN VARIANTS 1 AND 2 IN RAT SINGLE STRIATAL CHOLINERGIC INTERNEURONS**

**Smith L.M.**, Oswald M.J., Stanton J.L., Reynolds J.N.J. and Clements K.M.

Department of Anatomy and Structural Biology, School of Medical Sciences, University of Otago, Dunedin, New Zealand.

Tonically active striatal cholinergic interneurons exhibit prominent afterhyperpolarisations following depolarising events. Hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels play a role in determining the length of this afterhyperpolarisation. HCN channels are nonspecific cation channels involved in control of resting membrane potential. Four variants of this channel have been described (HCN1-4) but it is not known which of these are expressed in cholinergic interneurons. We tested for the presence of HCN1 and HCN2, which have been detected in the rat striatum by immunohistochemical techniques. Our aim was to develop a reverse transcription-polymerase chain reaction (RT-PCR) protocol to detect specific HCN mRNA molecules in single neurons. To extract the cell contents, single cholinergic interneurons in striatal rat brain slices were morphologically identified by their large somata and thick primary dendrites. Whole-cell patch-clamp recordings were conducted to confirm the characteristic electrophysiological signature of these neurons. The cytoplasmic contents were extracted by applying gentle suction through the patch pipette, before being processed for detection of HCN1 or HCN2 mRNA expression, using nested PCR techniques. Expression of choline acetyltransferase (ChAT), an enzyme only expressed in cholinergic interneurons, was also examined. mRNA in the extracted cytoplasmic contents of nine interneurons was investigated. Negative and positive controls were run with the cell extracts, and identified false positive results were discounted. ChAT was detected in most extracts examined (6/8), HCN2 in some (2/5), and HCN1 in none (0/5). We are currently optimising protocols to improve ChAT detection and utilising real-time PCR to improve the sensitivity for detecting and quantifying low levels of HCN mRNA expression.

## POS-MON-075

**A HETEROGENEOUS STOICHIOMETRY OF  $\alpha 9\alpha 10$  NICOTINIC ACETYLCHOLINE RECEPTORS IS DETECTED BY THE SELECTIVE CONOTOXIN VC1.1**

**Absalom N.L.**<sup>1</sup>, Liang G.<sup>1</sup>, Pera E.<sup>1</sup>, Chu C.<sup>1</sup>, Kim H.-L.<sup>1</sup>, McIntosh J.M.<sup>2,3</sup> and Chebib M.<sup>1</sup>

<sup>1</sup>Faculty of Pharmacy, University of Sydney, Camperdown, NSW 2006. <sup>2</sup>Department of Psychiatry, University of Utah, Salt Lake City, Utah 84112. <sup>3</sup>Department of Biology, University of Utah, Salt Lake City, Utah 84112.

Nicotinic acetylcholine (nACh) receptors are ligand-gated ion channels involved in fast synaptic transmission. nAChRs are pentameric complexes formed from combination of alpha and beta subunits, with the  $\alpha 9\alpha 10$  heteromeric complex found in inner hair cells, dorsal root ganglion neurons and lymphocytes. The  $\alpha 9\alpha 10$  receptor has previously been reported to form a stoichiometry of  $(\alpha 9)_2(\alpha 10)_3$ . The conotoxins Vc1.1 and RglA are potent and selective inhibitors of acetylcholine-evoked currents in  $\alpha 9\alpha 10$  receptors. We have investigated the stoichiometry of  $\alpha 9\alpha 10$  receptors by conotoxin inhibition of ACh-evoked currents recombinantly expressed in *Xenopus* oocytes. We show that Vc1.1 inhibit ACh-evoked currents in a biphasic inhibition curve. We show that the characteristics of this curve can be altered by varying the ratio of  $\alpha 9$  and  $\alpha 10$  RNA injected into the oocytes from 1:1 to 10:1  $\alpha 9:\alpha 10$  ( $n \geq 3$  for each ratio). Furthermore, the biphasic nature of the curve is almost completely removed by "flooding" the injection ratio with  $\alpha 10$  subunits at a ratio of 1:3  $\alpha 9:\alpha 10$ . We interpret these results as demonstrating that the conotoxin Vc1.1 does not inhibit ACh-evoked currents when binding at the  $\alpha 9-\alpha 10$  and  $\alpha 9-\alpha 9$  interfaces in an equivalent manner and that the biphasic nature of the curve is a result of a mixed population of the receptors, in contrast to inferred stoichiometry using agonist-evoked concentration-response curves. We conclude that the receptor can form in either the  $(\alpha 9)_2(\alpha 10)_3$  or the  $(\alpha 9)_3(\alpha 10)_2$  stoichiometry *in vitro*.

## POS-MON-074

**MODULATION OF THE  $Ca^{2+}$  CONDUCTANCE OF NICOTINIC ACETYLCHOLINE RECEPTORS BY THE ENDOGENOUS PROTEIN LYPD6**

**Morsch M.**<sup>1,3</sup>, Darvas M.<sup>2</sup>, Racz I.<sup>2</sup>, Zimmer A.<sup>2</sup>, Ahmadi S.<sup>1</sup> and Swandulla D.<sup>1</sup>

<sup>1</sup>Institute of Physiology, University of Bonn, Germany <sup>2</sup>Institute of Molecular Psychiatry, Life & Brain Center, University of Bonn, Germany <sup>3</sup>Institute of Physiology and Bosch Institute, University of Sydney, Australia

The agonist binding sensitivity and desensitisation kinetics of nicotinic acetylcholine receptors (nAChRs) can be modulated by snake venom neurotoxins and related endogenous small proteins of the uPAR-Ly6 family. We have identified Lypd6, a distantly related member of this family as a modulator of nAChRs in neurons. Transgenic mice overexpressing Lypd6 display behaviors that were indicative of an enhanced cholinergic tone, such as a higher locomotor arousal and hypoalgesia. These mice are also more sensitive to the analgesic effects of nicotine. In trigeminal ganglia cells Lypd6 selectively enhanced the  $Ca^{2+}$ -component of nicotine evoked currents through nAChRs, as evidenced by comparative whole-cell patch clamp recordings and  $Ca^{2+}$ -imaging. In contrast, a knockdown of Lypd6 expression using siRNAs selectively reduced nicotine-evoked  $Ca^{2+}$ -currents. Pharmacological experiments with blockers such as alpha-Bungarotoxin or methyllycaconitine revealed that the nAChRs involved in this process are heteromers. Taken together, Lypd6 seems to constitute a novel modulator of nAChRs that affects receptor function by selectively increasing  $Ca^{2+}$ -influx through this ion channels.

## POS-MON-076

**INVESTIGATING THE NICOTINIC NON-COMPETITIVE BINDING SITE**

**Quek G.X.J.**<sup>1</sup>, Halliday J.I.<sup>2</sup>, Absalom N.L.<sup>1</sup>, McLeod M.D.<sup>2</sup> and Chebib M.<sup>1</sup>

<sup>1</sup>The University of Sydney. <sup>2</sup>The Australian National University.

Novel nicotinic acetylcholine receptor (nAChR) antagonists have been derived from methyllycaconitine (MLA). AE Succinimide analogue [(3-ethyl-9-methylene-3-aza-bicyclo[3.3.1]nonan-1-yl)methyl-2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate] contains an anthranilate ester side-chain displaying mixed competitive binding on  $\alpha 4\beta 2$ ,  $\alpha 3\beta 4$  nAChRs and competitive binding  $\alpha 7$  nAChRs. Radioligand binding studies and X-ray crystallography has provided strong evidence that non-competitive binding sites exist at non- $\alpha$  interfaces of heteromeric nAChRs. Here we report studies of the non-competitive binding site of the AE Succinimide analogue within the channel pore and the N-terminal domain on the  $\alpha 4\beta 2$  nAChR. Water accessibility of the residues was first examined using the Substituted Cysteine Accessibility Method (SCAM). Loop A (D115, V116, V117, L118, Y119, N120) of the  $\beta 2$  subunit, Loop D (N88, V89, W90, V91, K92, Q93, E94) of the  $\alpha 4$  subunit and known accessible channel residues (2' T278, 6' S282, 9' L285, 13' V289, 16' L292) of the  $\alpha 4$  subunit were individually mutated to cysteine, expressed in *Xenopus* oocytes and analysed using two-electrode voltage clamp recordings. Surface accessibility was evaluated by the reaction of sulfhydryl reagent ethylammonium-methanethiosulfonate (MTSEA) in the opened (in the presence of ACh) and closed channel states (in the absence of ACh). The site was then evaluated using two methods: (1) The antagonists were competed with the sulfhydryl reagents where protection from irreversible inhibition infers the binding site (2) AE Succinimide analogue was synthesized into a thiol reactive probe capable of reacting with cysteine directly. All mutants generated functional receptors and most were accessible to MTSEA. Both competition and reactive probe experiments showed that AE Succinimide analogue does not bind within the N-terminal domain but binds within the channel in the 13'V289 and 16'L292 position. Other loops within the non-competitive  $\beta(-)\alpha(+)$  interface and the competitive  $\alpha(+)\beta(-)$  interface will be studied in the future.

## POS-MON-077

**EFFECTS OF LOBELINE, A NICOTINIC RECEPTOR LIGAND, ON THE CLONED CARDIAC K<sup>+</sup> CHANNELS, Kv1.5, Kv3.1 AND Kv4.3**Jeong I.J.<sup>1</sup>, Hahn S.J.<sup>1</sup> and Choi B.H.<sup>2</sup><sup>1</sup>Department of Physiology, Medical Research Center, College of Medicine, The Catholic University of Korea, Seoul 137701, Republic of Korea. <sup>2</sup>Department of Pharmacology, Chonbuk National University, Jeonju, Republic of Korea.

The effects of lobeline, an agonist at nicotinic receptors, on Kv1.5, Kv3.1 and Kv4.3 stably expressed in CHO cells were examined using the whole-cell patch-clamp methods. Lobeline accelerated the decay rate of Kv1.5 inactivation, decreasing the current amplitude at the end of the pulse in a concentration-dependent manner with an IC<sub>50</sub> value of 15.11  $\mu$ M (n=7). The apparent binding ( $k_{+1}$ ) and unbinding rate ( $k_{-1}$ ) constants were  $2.43 \pm 0.22 \mu\text{M}^{-1}\text{s}^{-1}$  and  $40.92 \pm 11.57 \text{s}^{-1}$ , respectively (n=7). The calculated KD value derived by  $k_{-1}/k_{+1}$  was 16.86  $\mu$ M. Lobeline slowed the deactivation time course (n=8), resulting in a tail crossover phenomenon. The inhibition of Kv1.5 by lobeline steeply decreased at potentials between -20 and +10 mV, which corresponds to the voltage range of channel activation (n=7). At more depolarized potential, a weaker voltage-dependence was observed with a value of electrical distance ( $\delta$ ) of 0.26. Lobeline had no effect on the steady-state activation (n=5) but shifted the steady-state inactivation curves of Kv1.5 in the hyperpolarizing direction (n=7). Lobeline produced use-dependent inhibition of Kv1.5 at a frequency of 1 Hz and 2 Hz (n=9) and slowed the recovery from inactivation (n=5). Lobeline also inhibited Kv3.1 and Kv4.3 in a concentration-dependent manner with an IC<sub>50</sub> value of 21.76  $\mu$ M (n=6) and 28.25  $\mu$ M (n=6), respectively. These results indicate that lobeline blocks Kv1.5 by binding to the open state of the channels.

## POS-MON-078

**IRCINIALACTAMS: A NEW CLASS OF SUBUNIT-SELECTIVE GLYCINE RECEPTOR MODULATORS**Islam R.<sup>1</sup>, Balansa W.<sup>2</sup>, Fontaine F.<sup>2</sup>, Webb T.I.<sup>1</sup>, Gilbert D.L.<sup>1</sup>, Piggott A.M.<sup>2</sup>, Zhang H.<sup>2</sup>, Capon R.<sup>2</sup> and Lynch J.W.<sup>1</sup><sup>1</sup>QLD Brain Institute and. <sup>2</sup>Institute for Molecular Biosciences, University of QLD, Brisbane QLD 4072.

**Purpose:** The Glycine receptor (GlyR) chloride channel mediates inhibitory neurotransmission in the spinal cord, brain stem and retina. These receptors are not currently targeted by any therapeutic compounds. However, glyRs have emerged as possible targets for treating chronic inflammatory pain, temporal lobe epilepsy, tinnitus and spasticity. This study reports a novel compound class with strong GlyR subunit-specific actions that could eventually be useful as pharmacological probes or therapeutic leads. **Methods:** Extracts from >2500 southern Australian and Antarctic marine organisms were screened against  $\alpha 1$  and  $\alpha 3$  GlyRs stably expressed in HEK293 cells using an anion-sensitive yellow fluorescent protein assay. The potencies of novel pure compounds present in active fractions were quantitated at  $\alpha 1$  and  $\alpha 3$  GlyRs by automated patch-clamp electrophysiology. **Results:** This identified three Irciniidae sponges that yielded new examples of a rare class of glycyl lactam sesterterpene, ircinialactam A, 8-hydroxyircinialactam A, 8-hydroxyircinialactam B, ircinialactam C, ent-ircinialactam C and ircinialactam D. Structure activity relationship (SAR) investigations defined a new pharmacophore with potent and subunit selective modulatory properties against  $\alpha 1$  and  $\alpha 3$  GlyR isoforms. One compound, strongly potentiated  $\alpha 1$  GlyRs with an EC<sub>50</sub> of  $1.2 \pm 0.2 \mu\text{M}$  but inhibited  $\alpha 3$  GlyRs with an IC<sub>50</sub> of  $7.0 \pm 0.5 \mu\text{M}$  (both n = 5 cells). Such GlyR modulators may have potential application as pharmacological tools, and possibly as leads for the development of GlyR targeting therapeutics for chronic inflammatory pain, epilepsy, spasticity and tinnitus.

## POS-MON-079

**COMPARISON OF LIGAND-INDUCED CONFORMATIONAL CHANGES IN GLYCINE RECEPTOR  $\alpha 1$ ,  $\alpha 3$  AND  $\beta$  SUBUNIT M2 DOMAINS**

Han L.H., Wang Q.W. and Lynch J.L.

QLD Brain Institute and School of Biomedical Science, University of QLD, Brisbane, QLD 4072.

Understanding glycine receptor (GlyR) activation mechanisms is key to understanding their physiological and pharmacological properties. The pore-lining M2 transmembrane domain must move to open the channel, and the M2-M3 linker is important for initiating this movement. We have previously employed voltage clamp fluorometry (VCF) to monitor ligand-specific conformational changes at the 19' position in the  $\alpha 1$  GlyR M2-M3 domain. VCF involves tethering a rhodamine fluorophore to introduced cysteines and monitoring fluorescence and current changes during activation. In the present study we sought to determine whether the  $\alpha 3$  and  $\beta$  subunits responded in a similar way to the  $\alpha 1$  subunit during activation. GlyRs comprising mutated  $\alpha 1/\beta$ , and  $\alpha 3/\beta$  or  $\alpha 3$  subunits were expressed in *Xenopus* oocytes and studied using simultaneous voltage-clamp and micro-fluorometry. Oocytes were surgically removed from anaesthetized frogs by procedures approved by the University of QLD Animal Ethics Committee. During glycine activation, the  $\alpha 3$ -R19'C GlyR exhibited a dramatically decreased glycine sensitivity (EC<sub>50</sub> ~12 mM; all results averaged from  $\geq 5$  cells). In contrast,  $\alpha 1/\beta$ -R19'C GlyR exhibited wild type-like glycine sensitivities. Fluorescence of the label attached to  $\alpha 3$ -R19'C GlyR increased by ~7% and the glycine fluorescence and current dose-responses overlapped. However, unlike  $\alpha 1$  GlyR, the fluorescence response was slow to return to baseline after glycine removal. While fluorescence of  $\alpha 1/\beta$ -R19'C GlyR reached ~9% at saturating glycine concentrations, fluorescence dose-responses were right-shifted relative to current. Our results suggest that conformational changes experienced by  $\alpha 3$  and  $\beta$  subunit 19' residues are different to those experienced by the  $\alpha 1$  subunit. This suggests distinct conformation rearrangements during gating. We are currently seeking to understand the structural basis of these differences.

## POS-MON-080

**ETHANOL AND G $\beta$ y MODULATION OF THE  $\alpha 1$  GLYCINE RECEPTOR**

Jones S.L., Carland J.E. and Moorhouse A.J.

School of Medical Sciences, The University of New South Wales, Sydney 2052, Australia.

Glycine receptors (GlyRs) belong to the Cys-loop family of ligand gated channels and are important for fast neurotransmission in the central nervous system. GlyRs are potentiated by physiological concentrations of ethanol (<100 mM). Recently, it has been reported that this ethanol modulation of GlyRs occurs in a manner dependent on G-protein  $\beta$  subunits (G $\beta$ y). G $\beta$ y dimers have been shown to directly potentiate GlyR function via binding to the M3-M4 intracellular loop. In the current study, we established a reliable *in vitro* model of ethanol and G $\beta$ y modulation of human  $\alpha 1$  homomeric GlyRs ( $\alpha 1$ GlyRs) expressed in HEK293 cells. Intracellular dialysis of 0.5 mM GTP $\gamma$ S during whole-cell recordings potentiated a glycine EC<sub>10</sub> response by  $119 \pm 24\%$  (n=5), with responses to a maximal glycine concentration remaining unaffected. This indicates that G $\beta$ y modulation of GlyRs results in a leftward shift in the glycine concentration-response relationship. Co-application of 100 mM ethanol reversibly potentiated the response to an EC<sub>10</sub> glycine concentration by  $36 \pm 2\%$  (n=3). Interestingly maximal potentiation of  $\alpha 1$ GlyRs by GTP $\gamma$ S dialysis, did not occlude subsequent ethanol potentiation; co-application of 100 mM ethanol potentiated EC<sub>10</sub> glycine responses after dialysis by  $63 \pm 11\%$  (n=3), which was not significantly different to the extent of potentiation observed without exogenous activation of G-proteins. Preliminary data investigating the molecular basis of ethanol modulation indicate that mutation of both lysine residues within one of the proposed G $\beta$ y binding motifs, 385KK386, to glutamate, abolishes ethanol potentiation ( $2 \pm 7\%$ ; n=3). Overall, our data support an interaction between the structural determinants of both G $\beta$ y and ethanol modulation of  $\alpha 1$ GlyRs but also suggest that ethanol modulation is more complex than simply liberating G $\beta$ y subunits.

## POS-MON-081

**CHARACTERISING A HIGH-THROUGHPUT ASSAY FOR DRUG SCREENING AT THE  $\alpha 2\beta\gamma 1$  GABA<sub>A</sub> RECEPTOR**

Dixon C.L., Sah P. and Lynch J.W.

QLD Brain Institute and School of Biomedical Sciences, University of QLD, Brisbane QLD 4072.

The GABA<sub>A</sub> receptor is an important target for sedative and anxiolytic drugs. Recent work from our lab has shown that the  $\gamma 1$  subunit is highly expressed in the amygdala, and we propose that  $\gamma 1$ -containing receptors play an important role in anxiety. To investigate this hypothesis, we wish to identify selective modulators of  $\gamma 1$ -containing receptors. To achieve this, we intend to screen a library of compounds using an automated, fluorescence-based assay, which was developed in our lab (Gilbert *et al.*, 2009). The assay uses an iodide-quenchable yellow fluorescent protein (YFP-I125L), which is co-expressed with GABA<sub>A</sub> receptor subunits in HEK-293 cells. Cells are imaged and drug is added automatically. When the channels are opened by the addition of GABA in iodide-containing ringers solution, iodide influx is visualised as quench of the YFP. Activity in an unknown compound is suggested by a change in this quench. To ensure that the expressed channels contained the  $\gamma 1$  subunit, we used zinc, a selective antagonist of  $\gamma$ -containing receptors. Three different ratios of  $\alpha 2:\beta 2:\gamma 1$  DNA were trialled (1:1:3, 1:1:6, 1:1:9), as well as  $\alpha 2:\beta 2$  (1:1). Addition of zinc (100 $\mu$ M) reduced the quench in response to 10 $\mu$ M GABA by 94% in cells expressing  $\alpha 2:\beta 2$ , compared to 44% in cells expressing  $\alpha 2:\beta 2:\gamma 1$  (1:1:3). This difference was significant ( $p < 0.05$ , ANOVA with Dunn's multiple comparison), however increasing the quantity of  $\gamma 1$  DNA did not result in further resistance to Zn inhibition ( $p > 0.05$ ). Data are from at least 14 wells (containing at least 100 cells each) from 2 separate transfections. We conclude that this screen is an effective technique for identifying antagonists of  $\alpha 2\beta\gamma 1$  GABA<sub>A</sub> receptors.

## POS-MON-083

**INVESTIGATING GABA-A RECEPTOR PORE CONFORMATIONS USING DISULFIDE TRAPPING**

Yang Z., Webb T.L., Lynagh T.L. and Lynch J.W.

QLD Brain Institute, University of QLD, Brisbane, QLD 4072.

We previously employed a disulfide trapping approach in an attempt to determine how the pore-lining second transmembrane domains (M2) of  $\gamma$ -aminobutyric acid type A receptors (GABAARs) move to open the channel. The M2 domain T6' residue lines the pore, and we showed that  $\alpha 1/\beta 1T6'C$  receptors form 6' cysteine-mediated disulfide bonds in the closed. However, because GABA induced fast desensitization, investigating dimer formation in the open state was not possible. The present study addressed this by using the non-desensitising agonist, ivermectin, to induce a stable open state, thereby allowing comparison of M2 domain orientations in closed and open states. Patch-clamp electrophysiology and Western-blotting were both performed on GABAARs expressed in HEK293 cells. Whereas unmutated GABAARs were not locked open by ivermectin,  $\alpha 1/\beta 1T6'C$  GABAARs were locked open via disulfide bond formation. This was confirmed using both electrophysiology ( $n > 10$  cells) and Western blot ( $n = 3$ ). Also, a reducing agent, dithiothreitol, reduced the closed-state dimer but not the open-state dimer ( $n = 5$  cells each). Moreover, the closed state dimer needed to be reduced to enable formation of the open state dimer. We propose that, in both the closed and open states,  $\beta$  subunit 6' cysteines move into sufficiently close proximity for disulfide formation via large random motions that appear to be a unique feature of  $\beta$  subunits. Because cross-linking of adjacent  $\beta$  subunits prevents the channels from both opening and closing, a movement of adjacent subunits relative to one another must be essential for channel gating. Our results place constraints on the closed and open state structures of the GABAAR pore and provide evidence for the relative movement of  $\beta$  subunits during gating.

## POS-MON-082

**EFFECT OF GINKGO TERPENOID LACTONES ON CYSTEINYL MUTANTS OF GABA<sub>A</sub> RECEPTOR PORE**

Ng C.C., Duke R., Hinton T. and Johnston G.A.R.

Department of Pharmacology, Bosch Building, The University of Sydney, NSW, 2006, Australia.

Clinical studies showed that the extract of *Ginkgo biloba* (EGb 761) reduced anxiety without causing sedation<sup>1</sup>. Anxiolysis without sedation of the extract active constituents bilobalide and ginkgolide A was also demonstrated in animal models<sup>2,3</sup>. Anxiolytics and anticonvulsants positively modulate the action of GABA, whereas the convulsants (including chloride channel blocker picrotoxinin) negatively modulate the action of GABA. Bilobalide and ginkgolides are structurally similar to picrotoxinin, and like picrotoxinin, they have been shown to negatively modulate the action of GABA at  $\alpha 1\beta 2\gamma 2$  GABA<sub>A</sub> receptors. However, unlike picrotoxinin, bilobalide and ginkgolides are not known to cause convulsions. This study aims to identify possible differences in their activities by investigating their effects on the pore facing residues inferred to bind picrotoxinin. The residues at position 2', 6' and 15' of  $\alpha 1$ ,  $\beta 2$  and  $\gamma 2$  subunits were mutated one at a time to cysteine and co-expressed with the wild type subunits in *Xenopus* oocytes. This was carried out using 2-electrode voltage clamp electrophysiology. Compared to the wild type, all  $\beta 2$  mutants were 2 to 8 fold less sensitive to bilobalide and ginkgolide B whereas the  $\alpha 2'$  mutant was 11 fold more sensitive to ginkgolide A ( $n = 3-7$ ). The  $\beta 2'6'$  mutant was found to be 22 fold less sensitive to picrotoxinin ( $n = 6$ ). The lack of convulsant effects of bilobalide, and ginkgolide A and B may be associated in part with their different binding locations within the chloride channel. References 1. Woelk H *et al* (2007) *J Psychiatr Res*, 41, 472-480 2. Nolder M *et al* (2000) US patent 6022889 3. Kuribara H *et al* (2003) *J Nat Prod*, 66, 1333-1337.

## POS-MON-084

**EXTRACELLULAR LOOPS 2 AND 4 OF GLYT2 ARE REQUIRED FOR N-ARACHIDONYL-GLYCINE INHIBITION OF GLYCINE TRANSPORT**

Edington A.R., Mckinzie A.A., Ryan R.M. and Vandenberg R.J.

Discipline of Pharmacology, School of Medical Sciences, University of Sydney, NSW, 2006, Australia.

Concentrations of glycine are regulated via the Na<sup>+</sup>/Cl<sup>-</sup>-dependent glycine transporters, GLYT1 and GLYT2. N-Arachidonyl glycine (NAGly) is an endogenous inhibitor of GLYT2 with no effect on GLYT1. We investigated whether extracellular loops 2 and 4 (EL2/4) are important for NAGly sensitivity between GLYT1 and GLYT2 and a series of related N-arachidonyl amino acids (NAAAs). Chimeras were constructed between GLYT1 and GLYT2 with their EL2 and/or EL4 regions switched. Point mutations of all GLYT2 EL4 residues which differed from GLYT1 were mutated to the corresponding residue in GLYT1. Transporters were tested for their sensitivity to GLYT2 inhibitors: NAGly, N-arachidonyl-L-alanine (NALA), N-arachidonyl-D-alanine (NADA) and N-arachidonyl- $\gamma$ -aminobutyric acid (NAGABA). Transport currents induced by application of glycine alone and in the presence of the NAAAs were measured ( $n \geq 5$ /transporter). GLYT2 (and not GLYT1) is inhibited by NAGly, NADA and NAGABA whereas GLYT2(GLYT1EL2) and GLYT2(GLYT1EL4) had reduced sensitivities. Interestingly, GLYT2 and GLYT2(GLYT1EL2) are inhibited by NALA whereas GLYT2(GLYT1EL4) is not. GLYT2R531L and GLYT2K532G had reduced sensitivity to NAGly ( $IC_{50}$ : 13 $\pm$ 2  $\mu$ M; 9 $\pm$ 1  $\mu$ M, respectively) compared to GLYT2 ( $IC_{50}$ : 3.4 $\pm$ 0.6  $\mu$ M) while GLYT2I545L had markedly reduced sensitivity to NAGly ( $IC_{50}$ : >30  $\mu$ M). GLYT2R531L, GLYT2K532G and GLYT2I545L also had reduced sensitivities to NALA ( $IC_{50}$ : 14 $\pm$ 1  $\mu$ M; 12 $\pm$ 1  $\mu$ M; and 21 $\pm$ 1  $\mu$ M, respectively) compared to GLYT2 ( $IC_{50}$ : 5.9 $\pm$ 0.7  $\mu$ M). In conclusion, EL2 and EL4 of GLYT2 are important in the selective inhibition of GLYT2 by NAGly, NADA and NAGABA while only EL4 of GLYT2 is required for NALA inhibition of transport. Key residues in GLYT2 EL4 required for NAGly and NALA sensitivity are R531, K532 and I545.



## POS-MON-085

## EAAT5 MEDIATES GLUTAMATE TRANSPORT IN MOUSE VESTIBULAR EPITHELIUM

Lim R.<sup>1</sup>, Kindig A.E.<sup>1</sup>, Lee A.<sup>2</sup>, Pow D.V.<sup>2</sup>, Callister R.J.<sup>1</sup> and Brichta A.M.<sup>1</sup>

<sup>1</sup>School of Biomedical Sciences and Pharmacy, The University of Newcastle. <sup>2</sup>UQ Centre for Clinical Research, The University of Queensland.

Synaptic transmission between hair cells and primary afferent fibres in the inner ear is mediated by glutamate. Type I vestibular hair cells are enveloped by calyx afferent terminals. The unusual geometry of the calyx, and the tonic release of glutamate by type I hair cells at this synapse means mechanisms must exist to clear glutamate from the synaptic cleft and prevent postsynaptic receptor desensitisation. **Immunofluorescence:** Vestibular organs and retina (control) were obtained from mice (overdosed with Ketamine 300 mg/kg), sectioned and incubated in primary antibodies against the glial glutamate-aspartate transporter (GLAST) and EAAT5. **RT-PCR:** Total RNA was extracted from retina and vestibular epithelium and the EAAT5 gene amplified using RT-PCR. Reaction products were separated on 1.5% agarose gel. **Results:** Immunolabelling of GLAST was confined to supporting cells of the vestibular epithelium as shown previously. Until now, the expression of EAAT5 has only been reported in the retina. Significantly, RT-PCR and immunolabelling of EAAT5 show expression in crista and utricle. Interestingly, immunofluorescence of EAAT5 shows expression in both type I and II vestibular hair cells, as well as calyx primary afferent terminals and fibres. **Conclusions.** EAAT5 is highly expressed in the mouse crista and utricle. Active uptake of glutamate by EAAT5 by both hair cells and primary afferent fibres may limit glutamate concentration in the synaptic cleft, thereby preventing glutamate receptor desensitisation. The expression of EAAT5 at tonically active glutamatergic synapses such as those in the vestibular epithelium, and retina suggests highly efficient glutamate uptake mechanisms have developed to maximise receptor sensitivity.

## POS-MON-086

## EXPLORING THE ROLE OF TM8 AS A KEY DOMAIN IN INFLUENCING THE FUNCTIONAL PROPERTIES OF HUMAN GLUTAMATE TRANSPORTERS

Sirivanta T.<sup>1</sup>, Conigrave A.<sup>2</sup>, Vandenberg R.J.<sup>1</sup> and Ryan R.M.<sup>1</sup>

<sup>1</sup>Pharmacology, Bosch Institute, University of Sydney, NSW 2006.

<sup>2</sup>Molecular and Microbial Biosciences, University of Sydney NSW 2006.

Human Excitatory Amino Acid Transporters (EAATs 1-5) are responsible for the synaptic clearance of extracellular glutamate and play a key role in preventing excitotoxic cell injury. The transport process is coupled to the co-transport of 3 Na<sup>+</sup>, 1H<sup>+</sup>, and the counter-transport of 1K<sup>+</sup>. In addition to transport, EAATs also possess a thermodynamically uncoupled chloride conductance which is activated upon binding of the substrate and sodium ions. In 2004, the crystal structure of the bacterial aspartate transporter, *Pyrococcus horikoshii* (GltPh) was solved and serves as a basis for understanding the structure and function of the EAATs. GltPh shares ~36% amino acid identity with the human EAATs and there is high conservation of regions thought to be important to the transport process. This project seeks to develop a structural model of the EAATs and explore the structural basis for the pharmacology of these transporters. The highly conserved c-terminal half of the transporters (HP1, HP2, TM7, TM8) contain residues that have been implicated in substrate and ion binding/translocation. In TM8, there is a six-amino acid residue motif found in GltPh that is not present in the EAATs. To determine the significance of this motif, EAAT1/GltPh and EAAT2/GltPh chimeras were constructed and expressed in *Xenopus laevis* oocytes. It was observed that the two chimeras exhibited similar substrate selectivity and affinity as their respective wild-types. Interestingly, the degree of chloride conductance was enhanced in the EAAT2 chimera. Transport of the poor substrate, 4-methylglutamate was supported by the EAAT2 chimera. These results suggest that the TM8 motif that is unique to GltPh does not affect substrate transport, but does impact poor substrate selectivity and chloride conductance in EAAT2.

## POS-MON-087

## IDENTIFICATION OF NEW VARIANT FORMS OF THE PHOTORECEPTOR GLUTAMATE TRANSPORTER EAAT5

Lee A. and Pow D.V.

The University of Queensland Centre for Clinical Research, Herston, QLD 4029.

EAAT5 is the predominant glutamate transporter used by photoreceptors in the retina to recover glutamate released by their synaptic terminals. EAAT5 is unusual in having a large chloride conductance, so that recovery of glutamate may be associated with feedback regulation of release of glutamate, by modifying the membrane potential of the synaptic terminals. Accordingly any changes in the biophysical properties of the EAAT5 that is expressed might influence the functional properties of photoreceptors. Examination of Western blots of rat retinal lysate using antibodies to the amino- and carboxyl termini of EAAT5 revealed, contrary to our expectations, several bands at differing molecular weights, suggesting that smaller variant forms of EAAT5 might exist. PCR analysis was performed using primers flanking the coding region of EAAT5. Multiple bands were identified. Bands were excised, inserted into plasmids, expanded in *E. coli* and 30 clones sequenced. We identified 6 forms of EAAT5, including the originally described full-length wild-type form. Five splice variant forms were identified, which skipped, either completely or partially various exons, including exon 3, exon 7 exon 8, exon 9 and exon 10. The exon 8- and exon 10-skipping forms generated frame shifts that should lead to truncated proteins. Exon-skipping forms of EAAT1 and EAAT2 have previously been implicated in human disease, so ongoing studies of EAAT5 include production of antibodies to the exon skip forms, analysis of transport properties and determination of expression profiles in the normal human retina, and in retinas with disease including macular degeneration.

## POS-MON-088

## THE PHYSIOLOGICAL ROLE OF AMINOPEPTIDASE N IN THE TRANSPORT OF AMINO ACIDS

Fairweather S.J. and Broer S.

Australian National University.

The brush border membrane of the intestinal epithelium contains peptidase enzymes and amino acid transporters that mediate the final stages of digestion and the transport of amino acids, respectively. One of the most important peptidases is Aminopeptidase N (APN) which hydrolyses N-terminal amino acids from small peptide chains. Likewise, one of the most crucial transporters is the Broad Neutral Amino acid Transporter 1 (B<sup>0</sup>AT1), responsible for the bulk of neutral amino acids absorbed in higher mammals. The aim of this project was to discover more about the functional and structural interactions between B<sup>0</sup>AT1 and APN by characterising them in the *Xenopus laevis* oocyte heterologous expression system and murine intestinal brush border vesicles. In particular, an investigation was conducted into how APN alters the kinetic activity of B<sup>0</sup>AT1, and the mechanism by which this occurs. It was first discovered that APN increases the rate of B<sup>0</sup>AT1 mediated transport between four- and five-fold ( $p \leq 0.001$ ). Subsequent kinetic analysis found there were two components to this increase in transport activity: an increase in the maximum rate ( $V_{max}$ ) of B<sup>0</sup>AT1 transport ( $p \leq 0.05$ ), and an increase in the substrate affinity ( $K_m^{app}$ ) of B<sup>0</sup>AT1 ( $p \leq 0.001$ ). The increase in maximum rate was shown to be due to an increase in surface expression of B<sup>0</sup>AT1, indicating APN functions as a facilitator of B<sup>0</sup>AT1 trafficking to the plasma membrane ( $n = 3$ ). The mechanism by which APN increases the substrate affinity was also investigated. It was discovered that amino acid binding by APN increases the local concentration of B<sup>0</sup>AT1 substrate ( $p \leq 0.001$ ). Furthermore, it was found that this increase in local substrate concentration was not due to channelling of substrate by APN into the extracellular binding site of B<sup>0</sup>AT1. Therefore, the natural diffusion of substrate released from the active site appears to be the cause of the local increase in substrate concentration and, hence, the increase in B<sup>0</sup>AT1 substrate affinity.

## POS-MON-089

**BIOLOGICAL ACTIVITY OF ALANINE-SUBSTITUTED ANALOGUES OF  $\alpha$ -CONOTOXIN Vc1.1 ON N-TYPE CALCIUM CHANNELS IN RAT SENSORY NEURONS**Callaghan B.P.<sup>1</sup>, Jensen J.<sup>2</sup>, Clark R.J.<sup>2</sup>, Craik D.J.<sup>2</sup> and Adams D.J.<sup>1</sup><sup>1</sup>Health Innovations Research Institute, RMIT University, Bundoora, VIC, 3083. <sup>2</sup>Institute for Molecular Biosciences, University of Queensland, Brisbane, Qld 4072.

$\alpha$ -Conotoxin Vc1.1 is a 16 amino acid disulfide peptide that is a selective antagonist of the  $\alpha 9\alpha 10$  nicotinic acetylcholine receptor (nAChR) subtype but has recently been shown to be a more potent inhibitor of N-type  $\text{Ca}^{2+}$  channel currents in dissociated neurons from rat dorsal root ganglia (DRG). The inhibition of N-type  $\text{Ca}^{2+}$  channel currents was blocked by inhibitors of  $\text{G}_{\text{ip}}$  and selective  $\text{GABA}_{\text{B}}$  receptor antagonists suggesting that Vc1.1 acted via  $\text{GABA}_{\text{B}}$  receptors (Callaghan et al., 2008, *J. Neurosci.* 28:10943-51). To further explore the structure-activity relationship for Vc1.1 inhibition of N-type  $\text{Ca}^{2+}$  channels in DRG neurons, the amino acids except the conserved cysteines contained in the sequence of Vc1.1 (Gly(1)-Ser(4)-Asp(5)-Pro(6)-Arg(7)-Asn(9)-Tyr(10)-Asp(11)-His(12)-Pro(13)-Glu(14)-Ile(15)), were sequentially replaced by Ala. These analogues have been characterised by NMR spectroscopy demonstrating that the structure of the peptide is not significantly changed (Halai et al., 2009, *J. Biol. Chem.* 284: 20275-84). The present study examined the activity of the Vc1.1 analogues on high voltage-activated  $\text{Ca}^{2+}$  channel currents in rat DRG neurons using the whole-cell patch clamp technique. Analogues that resulted in significant shifts to the right of the concentration-response relationship for inhibition of  $\text{Ca}^{2+}$  channel currents included S4A (n=4), N9A (n=16) and P13A (n=2). In contrast, analogues with the least effect compared to Vc1.1 were D11A (n=4), E14A (n=2) and I15A (n=2). Interestingly [N9A]Vc1.1 has been reported to be more potent than Vc1.1 at the  $\alpha 9\alpha 10$  nAChR whereas it is inactive at inhibiting N-type  $\text{Ca}^{2+}$  channel currents. These findings contribute to an improved understanding of the molecular basis for the  $\text{GABA}_{\text{B}}$  receptor-mediated inhibition of the N-type calcium channel current by Vc1.1.

## POS-MON-091

**ADENOSINE MODULATES THE EXCITABILITY OF LAYER II STELLATE NEURONS IN ENTORHINAL CORTEX THROUGH A1 RECEPTORS**

Li Y., Fan S. and Hu Z.

Departments of Physiology, Third Military Medical University, Chongqing 400038, China.

Stellate neurons in layer II entorhinal cortex (EC) provide the main output from the EC to the hippocampus. It is believed that adenosine plays a crucial role in neuronal excitability and synaptic transmission in the CNS, however, the function of adenosine in the EC is still elusive. Here, the data reported showed that adenosine hyperpolarized stellate neurons in a concentration dependent manner, accompanied by a decrease in firing frequency. This effect corresponded to the inhibition of the hyperpolarization-activated, cation nonselective (HCN) channels. Surprisingly, the adenosine-induced inhibition was blocked by  $3\mu\text{M}$  8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective A1 receptor antagonists (n=6), but not by  $10\mu\text{M}$  3,7-dimethyl-1-propargylxanthine (DMPX), a selective A2 receptor antagonists (n=6), indicating that activation of adenosine A1 receptor was responsible for the direct inhibition. In addition, adenosine reduced the frequency but not the amplitude of miniature EPSCs and IPSCs, suggesting that the global depression of glutamatergic and GABAergic transmission is mediated by a decrease in glutamate and GABA release, respectively. Again the presynaptic site of action was mediated by adenosine A1 receptors (n=6). Furthermore, inhibition of spontaneous glutamate and GABA release by adenosine A1 receptor activation was mediated by voltage-dependent  $\text{Ca}^{2+}$  channels and extracellular  $\text{Ca}^{2+}$ . Therefore, these findings revealed direct and indirect mechanisms by which activation of adenosine A1 receptor on the cell bodies of stellate neurons and on the presynaptic terminals could regulate the excitability of these neurons.

## POS-MON-090

**TEMPORAL AND SPATIAL EXPRESSION OF SODIUM CHANNEL ALPHA-SUBUNITS AND SPLICE VARIANTS IN THE DEVELOPING C57BL/6 MOUSE BRAIN**Gazina E.V.<sup>1</sup>, Richards K.L.<sup>1</sup>, Mokhtar M.B.C.<sup>1</sup>, Thomas E.A.<sup>1,2</sup>, Reid C.A.<sup>1</sup> and Petrou S.<sup>1,3</sup><sup>1</sup>Howard Florey Institute, The University of Melbourne, 3010, Victoria, Australia. <sup>2</sup>Department of Physiology, The University of Melbourne, 3010, Victoria, Australia. <sup>3</sup>Centre for Neuroscience, The University of Melbourne, 3010, Victoria, Australia.

Genes encoding sodium channel alpha-subunits *Scn1a*, *Scn2a*, *Scn3a* and *Scn8a*, are subject to alternative splicing of coding exons 5N and 5A and are expressed in the developing mammalian brain. While the functional role of these splice variants is unknown, evidence suggest the isoforms have different electrophysiological properties with implications for epilepsy and other disorders. AIM: Provide the first descriptive analysis of sodium channel alpha-subunit mRNA expression, exon 5 splicing and protein expression in the developing C57BL/6 mouse brain. METHODS: Total mRNA expression in cortex, hippocampus, thalamus and cerebellum of male mice (P0-P39; n=3 per age) was determined using quantitative real-time RT-PCR, and relative expression of exon 5 splice variants was determined using RT-PCR followed by isoform-specific enzymatic digestion. We used immunohistochemistry on whole-brain cryo-sections to determine regional distribution of Nav1.1, 1.2 and 1.6 encoded by *Scn1a*, 2a and 8a respectively. RESULTS: During early brain development mRNA expression levels for *Scn1a*, *Scn2a* and *Scn8a* increased, in contrast, *Scn3a* mRNA expression decreased. *Scn1a* mRNA contains only exon 5A, due to the absence of exon 5N in mouse *Scn1a* gene. At birth, only *Scn2a* mRNA contained higher or equal amounts of 5N compared to 5A isoform in most brain regions. 5N/5A ratios for each of the three mRNAs vary across brain regions, with cortex > hippocampus > thalamus > cerebellum. In all brain regions and for all three alpha-subunits, 5N/5A ratios gradually decreased with age, leveling at a value between 0.1 and 0.2. We found Nav1.1, 1.2 and 1.6 was detected in neurons in all brain regions and explicitly in axons. Nav1.2 was the predominant subunit detected in early brain development. CONCLUSION: Our findings suggest potential involvement of common factors in the alternative splicing of exon 5 for all transcripts, and that expression of these factors varies between brain regions and changes during development.

## POS-MON-092

**IDENTIFICATION OF A LOSS-OF-FUNCTION POLYMORPHISM IN THE HUMAN P2X4 RECEPTOR**Stokes L.<sup>1</sup>, Skarratt K.K.<sup>1</sup>, Gu B.J.<sup>1</sup> and Wiley J.S.<sup>2</sup><sup>1</sup>Sydney Medical School - Nepean, University of Sydney, Penrith NSW 2750. <sup>2</sup>Howard Florey Institute, Alan Gilbert Building, Carlton South VIC 3053.

The P2X4 receptor is a ligand-gated ion channel activated by extracellular ATP. The *P2RX4* gene lies adjacent to the highly polymorphic *P2RX7* gene on chromosome 12q24.3. To date four non-synonymous single nucleotide polymorphisms (SNPs) have been found in *P2RX4* however the functional effects associated with these mutations in the receptor are unknown. Site directed mutagenesis was used to introduce mutations into a GFP-tagged human P2X4 plasmid and functional P2X4 responses were measured using whole cell patch clamp electrophysiology in transfected HEK-293 cells. The Tyr 315>Cys mutation showed a dramatic loss-of-function with a response of only 10.9% of wild-type P2X4 receptors (p=0.0002, n=4-8 cells). This tyrosine residue is predicted to contribute to ATP binding in the extracellular domain and the Tyr 315>Cys mutant displayed a reduced sensitivity to ATP ( $\text{EC}_{50}$  of 192  $\mu\text{M}$  compared to wild-type P2X4  $\text{EC}_{50}$  of 5  $\mu\text{M}$ ). The Ala 6>Ser, Ile 119>Val and Ser 242>Gly mutations showed no significant difference in ATP sensitivity. We genotyped 200-500 Caucasian subjects at four SNPs in the *P2RX4* gene to determine allele frequencies and found the Tyr 315>Cys was rare with a frequency of 0.011 (n=416 subjects).

## POS-MON-093

### THE EFFECT OF AGING ON ASTROCYTE CONNEXINS IN THE RAT RETINA: IMPLICATIONS ON NUMBER, PLAQUE SIZE, PROTEIN EXPRESSION AND HEMICHANNEL HETEROGENEITY

Mansour H.<sup>1</sup>, Cole L.<sup>2</sup> and Chan-Ling T.<sup>1</sup>

<sup>1</sup>Discipline of Anatomy, School of Medical Sciences and Bosch Institute, University of Sydney, NSW 2006, Australia. <sup>2</sup>Advance Microscopy Facility, Bosch Institute, University of Sydney, NSW 2006, Australia.

Connexins (Cx) are a family of molecules intimately involved with cell communication. The aim of this study was to investigate changes in astrocyte connexins with respect to number, plaque size, protein expression and hemichannel heterogeneity during physiological aging. Astrocytes in retinal whole-mount preparations from Wistar rats aged 3 months (young adult), 9 months (middle-aged), and 22 months (aged), ( $n = 4$  per age examined), were analysed both qualitatively and quantitatively using Western blot analysis and immunofluorohistochemistry. Glial fibrillary acidic protein and *Griffonia simplicifolia* isolectin B4 were used to co-visualize astrocytes and blood vessels respectively. Our study revealed that Cx26, -30, -43, -45 are localised in astrocytes in the rat retina with Cx30 being the more dominant connexin expressed across all age groups. Interestingly, a significant increase in the number, plaque size and protein expression of Cx30 was observed both in parenchymal and vascular-associated astrocytes at 22 months compared to young adult rats. On the other hand, Cx43 number and plaque size in both astrocyte populations remained unchanged beyond 9 months of age. In comparison, Cx26 and Cx45 were expressed at lower levels than Cx30 and Cx43 during aging. Levels of Cx26 and Cx45 were found to increase from 3 to 9 months, followed by a decrease in the aged group. In addition, a significant increase in the number of heteromeric Cx26/Cx45 hemichannels was revealed during aging in both astrocyte populations. Similarly, Cx30/Cx43, Cx30/Cx45 and Cx43/Cx45 populations were all found to increase with age, but expressed at lower levels than Cx26/Cx45. In contrast, a reduction in the number of colocalised Cx26/Cx30 and Cx26/Cx43 was observed in both astrocyte populations with aging. Our novel findings will better comprehend the underlying function syncytium of astrocyte gap junctions in glial-neuronal-vascular interactions during physiological aging.

## POS-MON-094

### TRP CHANNELS DETERMINE HUMAN KERATINOCYTE DIFFERENTIATION: NEW INSIGHT INTO BASAL CELL CARCINOMA

Vandenbergh M., Lehenkyi V., Beck B., Prevarskaya N. and Skryma R. Laboratory of Cell Physiology, INSERM U800, University of Lille1, FRANCE.

Aberrant keratinocyte differentiation is considered to be a key mechanism in the onset of hyperproliferative dermatological diseases, including basal cell carcinoma (BCC). The role of calcium in keratinocyte differentiation is uncontested but the mechanisms controlling calcium-induced differentiation have yet to be completely elucidated. We studied the role of calcium-permeable TRP channels in human keratinocyte differentiation and BCC, using a combination of molecular and cell biology approaches, involving electrophysiology and  $Ca^{2+}$  imaging, on the HaCaT cell line, primary cultures of normal human keratinocytes, and BCC cells. We demonstrated that TRPC1/TRPC4 and TRPV6 channel expression was a "sine qua non" condition for keratinocyte differentiation, as knocking out these channels prevented the induction of  $Ca^{2+}$ -induced differentiation. TRPC1/TRPC4- and TRPV6- mediated calcium entries were significantly increased in differentiated keratinocytes. However, the failure of BCC cells to differentiate was related to a downregulation of TRP channels. In summary, our data demonstrate that TRP channels are key elements in keratinocyte  $Ca^{2+}$  homeostasis and differentiation and may therefore be responsible for skin pathologies.

## POS-MON-095

### THE AXONAL TRANSPORT AND RELEASE OF PROBDNF IS MEDIATED BY HUNTINGTIN ASSOCIATED PROTEIN-1 IN RODENT NEURONS

Yang M.<sup>1</sup>, Lim Y.<sup>1</sup>, Zhong J.H.<sup>1</sup>, Sun A.<sup>1</sup>, Wang Y.J.<sup>1</sup>, Li X.J.<sup>2</sup> and Zhou X.F.<sup>1</sup>

<sup>1</sup>Department of Human Physiology, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia. <sup>2</sup>Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA.

ProBDNF, a precursor of brain-derived neurotrophic factor (BDNF), is sorted into the secretory pathway, transported and released. However, the mechanism of its transport and release remains unclear. In this study, we report that Huntingtin associated protein-1 (HAP1) regulates proBDNF intracellular trafficking via the interaction with the prodomain of BDNF. The immunoprecipitation studies identified HAP1 as a cofactor associated with the prodomain in cotransfected HKE293 cells and rat brain lysate. Confocal imaging revealed that the colocalization (>90%) mostly occurred on vesicles, which were distributed in both soma and axons in transfected PC12 cells and cultured cortical neurons. Contrarily, proBDNF was only stained in soma of HAP1<sup>-/-</sup> neurons, suggesting that the lack of HAP1 leads to the redistribution of proBDNF and loss of proBDNF in secretory vesicles. This interaction was further confirmed by FRET, showing a high FRET efficiency (>20%) between HAP1A and the prodomain. Consistent with these studies, the anterograde and retrograde transport of proBDNF could be rescued by introducing HAP1 cDNA into mouse HAP1<sup>-/-</sup> neurons. The lack of HAP1 also abolished the activity-dependent release of proBDNF from cortical neurons. Immunostaining showed that HAP1 was recruited in vesicular proBDNF/ Golgi apparatus and associated with sortilin complex in cortical neurons. It is implied that HAP1 may be participated in sorting proBDNF into secretory vesicles via Golgi network. Taken together, our findings reveal that HAP1 plays an essential role in the axonal transport and release of proBDNF in cortical neurons.

## POS-MON-096

### MICROGLIA ARE ACTIVATED IN THE PARAVENTRICULAR NUCLEUS OF STZ DIABETIC RATS

Rana I.<sup>1,2</sup>, Badoer E.<sup>1,2</sup> and Stebbing M.J.<sup>1,2</sup>

<sup>1</sup>School of Medical Sciences. <sup>2</sup>Health Innovations Research Institute, RMIT University, Bundoora 3083, Vic.

Cardiovascular complications are common in diabetes, and include cardiomyopathy, hypertension, increased sympathetic nerve activity and increased risk of sudden cardiac death. Diabetes causes pathological changes in peripheral nerves and blood vessels. However, there is increasing evidence that inflammation within the central nervous system and dysregulation of sympathetic nerves play a role in diabetic complications. We previously reported that microglia (the brain's resident immune cells) are activated within the paraventricular nucleus (PVN) in rats with heart failure, a condition also associated with sympathetic dysregulation. We therefore investigated whether microglial activation occurred within central cardiovascular centres in several diabetes-related rat models. Brains were harvested from streptozotocin (STZ) diabetic rats 8-10 weeks after i.v. STZ administration ( $n=6$ ) or vehicle treated controls ( $n=5$ ), from rats fed a high fat diet ( $N=4$ ), from a strain of rats with low running capacity that were insulin resistant ( $N=3$ ), from Obese Zucker rats ( $N=3$ ) and from non-obese Zucker rats. Brains were immersion fixed and processed for immunohistochemistry using OX-42 antibody, a specific marker for microglia. Activated microglial cells were identified on the basis of intense OX-42 staining and on morphological criteria. Significantly increased numbers of activated microglia were seen in the PVN and the nucleus tractus solitarius of the brain stem in STZ rats. Microglia were not activated in other cardiovascular centres, in adjacent cortex or in these regions in any of the other rat strains studied. The pathological processes leading to microglial activation in STZ rats remain to be determined. It appears, however, that this activation is associated with overt diabetes, rather than insulin resistance or obesity.



## POS-MON-097

**EFFECT OF HYDROGEN SULPHIDE IN THE BRAIN ON CARDIOVASCULAR REGULATION**

Streeter E., Badoer E. and Favaloro J.  
RMIT, Bundoora, PO Box 71, Vic.

Hydrogen Sulphide gas has long been known for its smell and toxicity. In the last decade, however, hydrogen sulphide has been found to have several physiological effects including neuromodulatory roles, vasodilatory and cardioprotectant effects. More recently it has been suggested that hydrogen sulphide acts within the brain to reduce blood pressure. In the present study we have investigated the effects of microinjecting a hydrogen sulphide donor and the effects of inhibiting endogenous hydrogen sulphide production on blood pressure (BP), heart rate (HR) and lumbar sympathetic nerve activity (LSNA) in anaesthetised Wistar-Kyoto rats. We have concentrated on the paraventricular nucleus (PVN) in the hypothalamus and the pressor region of the rostral ventrolateral medulla (RVLM), areas known to have important cardiovascular regulatory functions. Rats were anaesthetised initially with inhaled isoflurane (1-3% in air), the femoral vein and artery were cannulated and the lumbar sympathetic nerve exposed and recorded. Anaesthesia was then maintained using urethane (1-1.5g/kg IV) with supplemental doses as required (0.1-0.3g/kg IV). The results show that bilateral microinjections (100nl/side) of either the hydrogen sulphide donor (NaHS, 20-2000pmol, n=5) or inhibitors of the enzyme which produces hydrogen sulphide (hydroxylamine (0.2-2nmol, n=5) or amino-oxyacetate (0.1-1nmol, n=5)) into the PVN did not significantly affect BP, HR and LSNA, compared to vehicle. In separate groups of rats, when NaHS (0.2-2000pmol, n=5), or the inhibitors, (as above), were microinjected bilaterally into the pressor region of the RVLM, no significant effect on BP, HR and LSNA was observed compared to vehicle controls. At the end of each experiment the injection sites in the brain were confirmed by histology. These results suggest that hydrogen sulphide in the hypothalamic PVN or the RVLM does not play a major role in the regulation of the cardiovascular system.

## POS-MON-099

**COMPARISON OF HEART RATE VARIABILITY IN SUBJECTS WITH PARKINSON'S DISEASE OR EXTRAPYRAMIDAL MOTOR SLOWING**

Brown R.<sup>1,2</sup>, Duma S.R.<sup>1</sup>, Broe G.A.<sup>1</sup> and Macefield V.<sup>1,2</sup>  
<sup>1</sup>Prince of Wales Medical Research Institute, Sydney, Australia.  
<sup>2</sup>School of Medicine, University of Western Sydney, Australia.

Parkinson's disease is a degenerative neurological condition, associated with dysfunction of the autonomic nervous system. Heart rate variability (HRV) is a non-invasive means of assessing autonomic control of the heart, and has been utilised in many studies involving Parkinson's disease. The purpose of our study was to compare participants with Parkinson's disease, extrapyramidal motor slowing, older healthy controls, and young healthy controls. Spectral analysis of HRV was assessed at rest and during 2 minutes of slow deep breathing in 97 participants. Low frequency (LF) HRV, believed to represent both sympathetic and parasympathetic cardiac activity, high frequency (HF) thought to represent parasympathetic activity, and low frequency/high frequency (LF/HF) ratio were measured. There were no differences in HRV between older healthy controls, extrapyramidal motor slowing, and Parkinson's disease. The only differences were seen between the young healthy controls and the three older groups. For resting activity, LF was lower, HF higher and the LF/HF ratio lower in the young healthy controls than the older groups. For 2 minutes of slow deep breathing, LF was higher, HF lower and the LF/HF ratio higher in the young healthy controls than the older groups. Given that there were no differences between participants with Parkinson's disease or extrapyramidal motor slowing, our results do not support the theory that HRV is a reliable indicator of autonomic dysfunction in Parkinson's disease. Moreover, that there were no differences between older healthy controls suggests that the changes in HRV seen in Parkinson's disease may simply be due to the normal aging process rather than the disease itself.

## POS-MON-098

**APOLIPOPROTEIN E GENOTYPE AND ASPECTS OF THE CARDIOVASCULAR RISK PHENOTYPE: IMPACT OF GENDER AND BODY WEIGHT (THE FINGEN STUDY)**

Kofler B.M.<sup>1</sup>, Miles E.A.<sup>2</sup>, Curtis P.<sup>3</sup>, Lietz G.<sup>3</sup>, Packard C.J.<sup>4</sup>, Caslake M.C.<sup>4</sup>, Mathers J.C.<sup>3</sup>, Williams C.M.<sup>1</sup>, Calder P.C.<sup>2</sup> and Minihane A.M.<sup>1,5</sup>  
<sup>1</sup>University of Reading, UK. <sup>2</sup>University of Southampton, UK. <sup>3</sup>University of Newcastle, UK. <sup>4</sup>University of Glasgow, UK. <sup>5</sup>University of Auckland, NZ.

ApoE genotype has been consistently associated with cardiovascular disease (CVD) risk, with an approximately 50% higher incidence in E4 carriers (25% Caucasians). Using a variety of cell and animal transgenic models we have previously reported a significantly higher inflammatory and pro-oxidant response associated with the ε4 allele (1). Here the impact of apoE genotype on inflammation and oxidative status in humans is reported. Data is taken from the baseline measurements of the FINGEN intervention trial, which examined the impact of modest dose fish oil intervention on over 40 CVD risk biomarkers in n=312 healthy UK adults, prospectively recruited on the basis of apoE genotype, age and gender. A significant impact of genotype was evident with 19% higher VCAM-1 (P=0.023), 32% lower P-selectin (P=0.004) and 13% higher oxidised LDL (P<0.001) evident in E4 carriers relative to the wild-type E3/E3 group. Furthermore, a significant impact of apoE genotype on C-reactive protein was observed (P=0.003) with the highest concentration in the E2 subgroup. Significant genotype x BMI interactions emerged, with the impact of genotype only evident in normal weight individuals (BMI 18.5-24.9kg/m<sup>2</sup>). The current data is indicative that the impact of apoE genotype on disease risk in humans may be in part attributable to its impact on inflammation and oxidative status. Further research is needed to gain insight into underlying mechanisms. <sup>1</sup> Minihane AM, et al., Apolipoprotein E genotype, cardiovascular risk and responsiveness to dietary fat manipulation. *Proceedings of the Nutrition Society* 2007;66:183-187.

## POS-MON-100

**MEASUREMENT OF ABSOLUTE AMOUNT OF CALSEQUESTRIN 2 PRESENT IN CARDIAC VENTRICULAR MUSCLE**

Murphy R.M.<sup>1</sup>, Mollica J.P.<sup>1</sup>, Beard N.A.<sup>2</sup> and Lamb G.D.<sup>1</sup>  
<sup>1</sup>Department of Zoology, La Trobe University, Victoria, 3086. <sup>2</sup>John Curtin School of Medical Research, Australian National University, Canberra City, ACT.

Calsequestrin 2 (CSQ2) is generally regarded as the primary calcium buffering molecule present inside the sarcoplasmic reticulum (SR) in cardiac cells, though its role as a calcium buffer has been questioned recently (Knollmann, J Physiol 587, 3081-3087, 2009). The aim of this study was to determine the absolute amount of CSQ2 present in cardiac ventricular cells, in order to gauge the likely influence of CSQ2 on the total and free calcium concentration within the SR. Whole hearts from freshly killed sheep were obtained from an abattoir. Ventricular tissue was homogenized (1:10) in Na-EGTA solution, and 5 to 10 µg samples loaded in their entirety and separated by 8% SDS-PAGE and CSQ2 detected by Western blotting, similar to our work with skeletal muscle (Murphy et al. J Physiol 587, 443-460, 2009). Intensities of the respective bands were compared to those obtained with various amounts (2.5 to 20 ng) of purified CSQ2 on the same blots. The fidelity of the quantification was verified by comparing signals from samples, purified CSQ2, and samples with added amounts of purified CSQ2. Ventricular tissue from n=8 sheep contained on average 23 ± 2 µmol CSQ2 per kg wet weight. Qualitative assessment of CSQ2 content by staining homogenate samples with Stains-All indicated that CSQ2 content of rat ventricular tissue was similar or even higher than that found in sheep heart. This amount of CSQ2 could bind a maximum of ~1 mmol calcium per kg of ventricular tissue, more than ample to account for current estimates of total SR calcium content of such tissue.

## POS-MON-101

**FUNCTION OF ADRENERGIC-STIMULATED CARDIAC RYRS**

Li J., Helden D. and Laver D.

School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia.

In cardiomyocytes, calcium is released from SR intracellular stores through ryanodine receptors (RyR). RyRs are regulated by  $\text{Ca}^{2+}$  in both the cytoplasm and SR lumen and their proper regulation plays an important role in cardiac output. Additionally, cardiac output is increased by stimulation of  $\beta$ -adrenergic receptors ( $\beta$ -AR) by adrenaline and noradrenaline.  $\beta$ -ARs couple to Gs $\alpha$ -protein, leading to phosphorylation of numerous targets including the RyRs. There are conflicting reports about how, and where, RyRs are phosphorylated in situ and there is no consensus on the effects of phosphorylation on RyR activity. Our objective is to understand how adrenergic-stimulation of cardiomyocytes influences the function of RyRs. Hearts were rapidly removed from adult male Sprague-Dawley rats and perfused with Krebs solution in a Langendorff apparatus (5 min). Hearts were then perfused (5 mins) with Krebs solution containing 1  $\mu\text{M}$  isoproterenol ( $\beta$ 1-adrenergic agonist) or with Krebs alone (control). Hearts were rapidly frozen in liquid N<sub>2</sub> and stored at -80°C. RyRs were isolated from these hearts and incorporated into artificial planar lipid bilayers and their activity was measured using single channel recording. RyRs (n=10) from control hearts were activated by both cytoplasmic and luminal  $\text{Ca}^{2+}$ . The mean activity of RyRs from isoproterenol-stimulated hearts was 10-fold higher than control RyRs at diastolic [ $\text{Ca}^{2+}$ ] (100 nM) but was not significantly different at systolic [ $\text{Ca}^{2+}$ ] (>1  $\mu\text{M}$ , n=19). Moreover, RyRs from stimulated hearts showed a bimodal distribution in activity with one population (12 out of 19) similar to RyRs from control hearts and another, excited population (7 out of 19) with reduced channel mean close times. Hence, adrenergic-stimulation changes the gating of RyRs in situ by increasing channel opening rates.

## POS-MON-102

**NIFEDIPINE-INSENSITIVE VASOCONSTRICTION OF PRESSURISED RAT BASILAR ARTERIES**

Ellis A. and Hill C.E.

John Curtin School of Medical Science, The Australian National University, Canberra, ACT, 0200.

Cerebrovascular constriction depends on influx of calcium through voltage dependent calcium channels (VDCCs). Although L-type channels are often attributed to this process, we have previously identified a role for nifedipine-insensitive VDCCs in regulating cerebrovascular tone in juvenile rats (Navarro-Gonzalez et al., 2009). Here we have extended these studies to adult rat basilar arteries, in which the effect of VDCC blockers was tested against vasoconstriction induced by intraluminal pressure, receptor activation (thromboxane mimetic, U46619; 0.1–1  $\mu\text{M}$ ) and depolarisation (10–120 mM  $[\text{K}^+]_o$ ). The selective L-type channel blocker, nifedipine (0.1, 1  $\mu\text{M}$ ), substantially inhibited pressure-induced constriction, whereas the putative T-type channel blocker, mibefradil, inhibited myogenic tone at low concentration (1  $\mu\text{M}$ ) but produced paradoxical constriction at high concentration (10  $\mu\text{M}$ ). A mibefradil analogue, NNC-550396 (3, 10  $\mu\text{M}$ ), inhibited constriction at both concentrations. However, effects of either mibefradil or NNC-550396 showed considerable overlap with nifedipine, indicating dual L and T actions of both inhibitors. Consequently, experiments were performed with sequential application of nifedipine (1  $\mu\text{M}$ ) followed by mibefradil (1  $\mu\text{M}$ ). Under these conditions, mibefradil caused additional relaxation over that produced by nifedipine (n=5,  $P < 0.001$ ), suggesting a component of tone that is resistant to L-type VDCC inhibition but sensitive to T-type channel inhibition. While U46619-induced constrictions were insensitive to nifedipine or mibefradil, constrictions to high  $[\text{K}^+]_o$ , that were not reliant on intracellular calcium (blocked by 10  $\mu\text{M}$  U73122), were reduced by nifedipine, leaving a small but significant residual component. We conclude that rat cerebral arteries employ both L- and T-type calcium channels to regulate vascular tone, however the non-specific actions of putative T-type channel blockers necessitate caution in their use when arguing for a role in vascular function. Navarro-Gonzalez, M.F. et al. (2009). Clin. Exp. Physiol. Pharmacol. 36, 55–66.

## POS-MON-103

**FRUCTOSE-FED MICE EXHIBIT MYOCARDIAL GROWTH AND CALCIUM HANDLING ABNORMALITIES ASSOCIATED WITH OXIDATIVE STRESS**Mellor K.M.<sup>1</sup>, Wendt I.R.<sup>2,1</sup>, Ritchie R.H.<sup>3</sup> and Delbridge L.M.D.<sup>1</sup><sup>1</sup>University of Melbourne. <sup>2</sup>Monash University. <sup>3</sup>Baker IDI Heart and Diabetes Institute.

Recent increase in the prevalence of insulin resistance has coincided with a marked elevation in dietary fructose intake. There is emerging evidence that insulin resistance impacts on the heart and the specific cardiac consequences of excess fructose intake require definition. The aim of this study was to determine the cardiac effects of a 12 week high fructose dietary intervention (60% energy intake) in C57Bl/6 male mice. Hyperglycemia (19% increase) and impaired glucose tolerance were observed coincident with normal plasma insulin levels. Hypertension and obesity were not contributing factors in this study. Hearts were collected for measurement of ventricular weight index (VWI) and myocardial production of superoxide (lucigenin chemiluminescence). Expression levels of signalling proteins (western blot) and cardiac hypertrophic genes (rtPCR) were analysed.  $\text{Ca}^{2+}$  handling (fura-2, 360:380nm ratio) and cell shortening (edge detection) properties of cardiomyocytes from fructose- and control-fed mice were analysed. A 22% increase in VWI in the fructose fed mice was associated with elevated superoxide production (fructose,  $553 \pm 28$  counts/sec/mg vs. control,  $489 \pm 11$  counts/sec/mg,  $p < 0.05$ ). Surprisingly, fructose fed mice exhibited suppressed expression of cardiac hypertrophic markers. Calcium transient amplitude was decreased in cardiomyocytes from fructose-fed mice associated with a slower calcium transient decay rate. Fructose feeding suppressed myocardial phosphorylation of Akt and S6. These findings demonstrate that a 12 week dietary fructose intervention induces cardiac hypertrophy associated with calcium handling dysregulation and oxidative stress. Specific signalling alterations may play a role in fructose induced cardiac pathologies. Further mechanistic studies are required to identify the basis of abnormal cardiac growth in this model.

## POS-MON-104

**THE ROLE OF STORE-OPERATED CALCIUM CHANNELS IN ENDOTHELIN-1-MEDIATED VASOCONSTRICTION OF RAT MESENTERIC ARTERIES**Chan Y.Y.<sup>1,2</sup>, Beltrame J.F.<sup>1,2</sup> and Wilson D.P.<sup>1,2</sup><sup>1</sup>University of Adelaide. <sup>2</sup>The Queen Elizabeth Hospital.

Cellular calcium is an essential regulator of vascular tone, which underscores the therapeutic potential of its regulation in the management of cardiovascular disease. Recent clinical and pharmacological evidence has indicated that the transient (T-type) calcium channels may be important in mediating endothelin-1 (ET-1) vasoconstriction. Using functional vascular myography, this study aimed to: (1) identify the efficacy of selective T-type calcium channel blockade (NNC 55-0396, 10  $\mu\text{M}$ ) compared to conventional L-type calcium channel blockade (verapamil, 10  $\mu\text{M}$ ) and (2) the contribution of intracellular inositol-1,4,5-trisphosphate-mediated calcium release and store-operated calcium entry to the activation of voltage-dependent calcium channels in ET-1-mediated vasoconstriction in isolated rat mesenteric arteries. Results indicated that the T-type calcium channel blocker, NNC 55-0396 is more effective than L-type calcium channel blocker, verapamil, in attenuating contractile responses in the context of  $\text{K}^+$ -mediated depolarisation (n=4) but not ET-1-mediated vasoconstriction (n=20). Inhibition of intracellular inositol-1,4,5-trisphosphate-mediated calcium release using the IP<sub>3</sub> receptor and store-operated calcium channel inhibitor, 2-aminoethyl diphenylborinate (100  $\mu\text{M}$ ) further attenuated the force ( $p < 0.05$ ; n=4). Following complete depletion of intracellular calcium using the sarcoendoplasmic reticulum calcium ATPase inhibitor, cyclopiazonic acid (10  $\mu\text{M}$ ), ET-1-mediated contractile responses were almost completely abolished ( $p < 0.05$ ; n=4). Combining calcium channels blockers with protein kinase C inhibitor (5  $\mu\text{M}$ ) also resulted in significant attenuation of ET-1-mediated vasoconstriction. In conclusion, extracellular, IP<sub>3</sub>-mediated and store-operated calcium channels, as well as PKC pathways are involved in ET-1-mediated vasoconstriction in the microvasculature. These data highlighted the importance of understanding the molecular mechanisms underlying the plethora of calcium entry pathways, as well as providing potential therapeutic targets to combat the detrimental effects of vasoconstriction for the management of cardiovascular disease.

## POS-MON-105

**RYANODINE RECEPTOR DYSFUNCTION IN ANTHRACYCLINE-INDUCED CARDIOTOXICITY**

Hanna A.D., Janczura M., Dulhunty A.F. and **Beard N.A.**  
Muscle Research Group, The John Curtin School of Medical Research, The Australian National University.

Anthracyclines are highly effective chemotherapeutic agents, used to treat various malignancies. However, their use is limited due to the onset of potentially fatal cardiotoxicity which presents with both acute and chronic complications. Current theories surrounding acute cardiotoxicity suggest synergistic effects due to accumulation in cardiomyocytes, where anthracyclines target sarcoplasmic reticulum protein(s), disrupting  $\text{Ca}^{2+}$  homeostasis. The cardiac  $\text{Ca}^{2+}$  release channel, the ryanodine receptor (RyR2), is thought to be modulated in part, by anthracycline-induced oxidation of critical sulfhydryl groups. In the present experiments, luminal (*trans*) addition of daunorubicin to RyR2 in lipid bilayers elicited a biphasic response, initially activating then inhibiting the channel. The initial daunorubicin-induced activation, but not the inhibition, was reversible with drug washout (N=8). The reducing agent dithiothreitol (DTT) prevented RyR2 inhibition, but not activation, consistent with an oxidation-induced inhibition process. Interestingly, DTT added to the cytoplasmic (*cis*) side of the chamber (but not the *trans* chamber) protected RyR2 from daunorubicin-induced inhibition, implying daunorubicin crosses the bilayer and oxidizes thiols in the cytoplasmic domain of RyR2, causing inhibition (N=8). DTT added after daunorubicin failed to reverse this anthracycline-induced inhibition (N=10), suggesting that upon oxidation, the modified thiols become buried within the RyR2 and inaccessible to DTT. The failure of DTT to prevent activation and washout-induced reversibility of activation suggest a ligand-binding mechanism, either to the RyR2, or an associated regulatory protein. Together these results implicate a high affinity ligand-binding action of anthracyclines on the RyR2 complex and that sulfhydryl oxidation is important in anthracycline-induced RyR2 inhibition. The results demonstrate that multiple mechanisms lead to anthracycline-induced disruption of RyR-dependent  $\text{Ca}^{2+}$  homeostasis and contribution to subsequent cardiotoxicity.

## POS-MON-107

**SPATIAL ASSOCIATION OF TRPC3,  $\text{IK}_{\text{Ca}}$  AND MYOENDOTHELIAL GAP JUNCTIONS IN RAT MESENTERIC ARTERY**

**Senadheera S.**, Kim Y. and Sandow S.L.  
University of New South Wales, Sydney, NSW 2052.

Sites of endothelial-smooth muscle cell close association (<30 nm) are integral for endothelium-dependent relaxation, and thus for control of blood flow and pressure. In rat mesenteric artery such specialized myoendothelial microdomain signalling sites consist of localized gap junction connexins (Cx), endoplasmic reticulum (ER) inositol 1,4,5-trisphosphate receptors, and intermediate conductance calcium-activated potassium channels ( $\text{IK}_{\text{Ca}}$ ). With previous data, such close spatial associations are consistent with potential for functional interaction. This study identifies a prospective channel responsible for ER calcium refilling at myoendothelial microdomain signalling sites in adult male SD rat mesenteric artery. Specificity of TRPC3 antibody against C' amino acids of mouse 822-835 TRPC3 (Alomone ACC-016; batches AN-02, 03, 07; AN-06 was non-specific), was characterized in fresh rat liver and HEK cells stably transfected with TRPC3 mouse cDNA using Western blotting and cell transfection, respectively. PCR amplification and sequencing verified the presence of transfected mouse TRPC3 gene transcript in HEK cells. Western blotting and confocal and ultrastructural immunohistochemistry determined the TRPC3 expression in rat mesenteric artery (n=3, for all experiments). Western blotting in liver confirmed antibody specificity with a faint ~98 kDa band that was partially blocked by peptide, and an apparent monoglycosylated band at ~120 kDa, which is recognized as the functional channel [1]; labelling for which was blocked by peptide. Antibody specificity was further confirmed by labelling transfected HEK cells, whilst untransfected cells failed to label. Western blotting confirmed monoglycosylated TRPC3 expression in rat mesenteric artery. Confocal and ultrastructural immunohistochemistry demonstrated TRPC3 localization at myoendothelial microdomains in close spatial association with  $\text{IK}_{\text{Ca}}$  and myoendothelial gap junction Cxs, consistent with potential for functional interaction. 1. Dietrich et al. J Biol Chem 2003; 278:47842-52.

## POS-MON-106

**FLECAINIDE BLOCKS  $\text{Ca}^{2+}$  RELEASE CHANNELS ASSOCIATED WITH CPVT-INDUCED CARDIAC ARRHYTHMIAS**

**Mehra D.**<sup>1</sup>, Van Helden D.F.<sup>1</sup>, Knollmann B.C.<sup>2</sup> and Laver D.R.<sup>1</sup>  
<sup>1</sup>Department of Biomedical Sciences and Pharmacy, University of Newcastle and HMRI, Callaghan, NSW 2308, Australia. <sup>2</sup>Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN, USA.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) causes sudden cardiac death due to mutations in the cardiac  $\text{Ca}^{2+}$  release channel (RyR2) or cardiac calsequestrin (CSQ2). Recently, it was shown that flecainide suppressed CPVT induced arrhythmias in humans and a CSQ2 null mouse model of CPVT by blocking RyR2. However, tetracaine, another classic RyR2 inhibitor, failed to do so because it caused excessive  $\text{Ca}^{2+}$  loading of the SR leading to pro-arrhythmic oscillatory  $\text{Ca}^{2+}$  release. RyRs were isolated from human and sheep hearts, and incorporated in artificial lipid bilayers to conduct single channel recordings under diastolic  $\text{Ca}^{2+}$  conditions. Flecainide (10  $\mu\text{M}$ , cytoplasmic) caused 50% inhibition of RyR2 by inducing subconductance states. In addition, flecainide decreased channel mean open time but had no significant effect on mean closed times. However, tetracaine (50% inhibition at 50  $\mu\text{M}$  cytoplasmic concentration) had no significant effect on mean open time but increased mean closed times. In CPVT mice ventricular myocytes, flecainide significantly reduced  $\text{Ca}^{2+}$  spark amplitude and spark width, resulting in a 40% reduction in spark mass. Surprisingly, flecainide significantly increased spark frequency. Consequently, flecainide had no significant effect on spark-mediated SR  $\text{Ca}^{2+}$  leak or SR  $\text{Ca}^{2+}$  content. In contrast, tetracaine decreased spark frequency and spark-mediated SR  $\text{Ca}^{2+}$  leak, resulting in a significantly increased SR  $\text{Ca}^{2+}$  content. We propose that smaller spark mass contributes to flecainide's antiarrhythmic action by reducing the probability of saltatory wave propagation between adjacent  $\text{Ca}^{2+}$  release units. Hence, RyR2 open-state inhibition provides a new therapeutic strategy to prevent diastolic SR  $\text{Ca}^{2+}$  waves and resulting triggered arrhythmia, like CPVT.

## POS-MON-108

**TAURINE SUPPLEMENTATION INCREASES RAT CARDIAC CALSEQUESTRIN 2 PROTEIN CONTENT, WHILE DECREASING THE TAURINE TRANSPORTER PROTEIN**

**Murphy R.M.**<sup>1</sup>, Horvath D.<sup>2</sup>, Stathis C.G.<sup>2</sup>, Hayes A.<sup>2</sup> and Goodman C.A.<sup>2,3</sup>  
<sup>1</sup>Department of Zoology, La Trobe University, Melbourne, 3086. <sup>2</sup>School of Biomedical and Health Sciences, Victoria University, Melbourne, 8001. <sup>3</sup>Department of Comparative Bioscience, University of Wisconsin, Madison, USA.

Taurine (Tau) is a conditionally essential beta-amino acid with diverse physiological roles. Tau supplementation has been used to treat a range of cardiac conditions, including congestive heart failure but mechanisms remain unclear. This study examined the effect of Tau supplementation on calcium handling protein contents and on the Tau transporter (TauT) protein in rat cardiac muscle. Twelve 8 wk Sprague Dawley rats were fed Tau (2.5% w/v) in drinking water *ad libitum* and standard chow for 2 wk while 10 rats (Con) were given normal drinking water and chow. Animals were killed by anesthetic overdose (Nembutal; ~85 mg/kg I.P.) in accordance with Victoria University Animal Ethics procedures and hearts rapidly dissected. There was no difference in body mass, whole heart or left ventricular masses, nor the amount of muscle water, as indicated by the left ventricle dry mass/wet mass ratio, between Con and Tau treated animals after supplementation. Tau supplementation resulted in an increase in total protein (Con 10.6±0.4 vs Tau 12.4±0.5  $\mu\text{g}$  protein/mg wet muscle,  $p=0.013$ ). Western blot analysis showed that Tau supplementation increased calsequestrin 2 protein (40%;  $p=0.005$ ) and decreased TauT protein (34%;  $p=0.0013$ ). There was no change in SERCA2, RyR2 or NCX proteins. In conclusion, Tau supplementation resulted in an increase total protein content and calsequestrin 2, which may help explain some of the benefits of Tau in heart failure. The observed decrease in TauT protein might suggest regulation of the total Tau content that the cardiac muscle can acquire.



## POS-MON-109

**DIFFERENTIAL EXPRESSION OF PACAP RECEPTORS IN THE ROSTRAL VENTROLATERAL MEDULLA DETERMINED BY QUANTITATIVE REAL-TIME PCR**

Lung M.S.Y., Farnham M.M.J. and Pilowsky P.M.

Australian School of Advanced Medicine, Macquarie University. Level 1 Dow Corning Building, 3 Innovation Road, North Ryde 2109, NSW Australia.

Pituitary adenylate cyclase activating polypeptide (PACAP) is an excitatory neuropeptide which is present in the central nervous system (CNS) in the form of a 38-amino acid peptide (PACAP-38). PACAP-38 caused sustained sympathoexcitation when microinjected into the rostral ventrolateral medulla (RVLM) of the rat (unpublished data), which is the primary centre for blood pressure control. The aim of this study was to determine the gene expression levels of the three G-protein-coupled receptors that PACAP acts on - PAC1, VPAC1 and VPAC2, by quantitative real-time polymerase chain reaction (qPCR), and to observe for differential expression between normotensive and hypertensive animals. Experiments were conducted on adult male Sprague-Dawley (SD; n=6), Wistar Kyoto (WKY; n=6) and Spontaneously Hypertensive (SHR; n=6) rats. The results show significant differences in the relative gene expression of the three receptors (2-Way ANOVA  $p < 0.0001$ ), with PAC1 being most abundant (SD=0.3931±0.03, WKY=0.3916±0.05, SHR=0.4047±0.05) followed by VPAC2 (SD=0.3293±0.07, WKY=0.1384±0.01, SHR=0.2234±0.03), then VPAC1 (SD=0.07035±0.026, WKY=0.07122±0.008, SHR=0.07994±0.019) which has the lowest level of expression in the RVLM. The relative level of PACAP receptors expression was similar across the three strains tested, with the exception of VPAC2 expression being significantly higher in SD compared to WKY (Bonferroni-adjusted t-test  $p < 0.01$ ). The findings of this study demonstrate the presence of all three PACAP receptors in the RVLM, and is consistent with reports of PAC1 being the predominant form in the CNS. The relative abundance of the PAC1, VPAC1 and VPAC2 receptor mRNAs do not differ between normotensive and hypertensive animals.

## POS-MON-111

**CATESTATIN ATTENUATES THE EFFECTS OF INTRATHECAL NICOTINE AND ISOPROTERENOL**Gaede A.H., Lung M.S.Y. and Pilowsky P.M.  
Macquarie University.

Catestatin (Cts; human chromogranin A<sub>352-372</sub>) is a neuropeptide derived from chromogranin A (ChgA). In the periphery it is released from the terminals of preganglionic neurons. In the adrenal medulla it inhibits catecholamine release by non-competitively antagonizing nicotinic cholinergic receptors. ChgA is present in the central nervous system, but the extent to which it is present within bulbospinal sympathoexcitatory neurons is unknown. We investigated the distribution of ChgA in the brainstem and its relationship to sympathoexcitatory neurons by combining immunofluorescence and *in situ* hybridization. A possible role for Cts in modulating the effect of other neurotransmitter systems in the spinal cord was examined by intrathecal injection of Cts, in conjunction with nicotine (1µg-100µg; n=5) and isoproterenol (0.12 µg – 2.5 µg; n=4), in the anaesthetised rat. Cts attenuated the hypotensive effect of isoproterenol on mean arterial pressure (maximum dose, 2.5 µg isoproterenol; -27 mmHg pre-Cts to -18 mmHg post-Cts), splanchnic sympathetic nerve activity (at 2.5 µg isoproterenol; 10.5% pre-Cts to 2.4% post-Cts), HR (at 2.5 µg isoproterenol; 1.1% pre-Cts to -1.6% post-Cts), and the dp/dt max of carotid pulse pressure (at 2.5 µg isoproterenol 17.3% pre-Cts to 9.3% post-Cts). Cts attenuated the hypertensive effect of nicotine on mean arterial pressure (at 10 µg nicotine, 19.3 mmHg pre-Cts to 6.8 mmHg post-Cts), splanchnic sympathetic nerve activity (at 10 µg nicotine, 10.7% pre-Cts to 4.5% post-Cts), and HR (at 10 µg nicotine, 4.1% pre-Cts to 2.0% post-Cts). The results indicate that Cts antagonizes both central nicotinic acetylcholine receptors and β-adrenoceptors that are involved in cardiovascular regulation *in vivo*.

## POS-MON-110

**DIMINISHED CARDIOVASCULAR BUT NOT EMOTIONAL REACTIVITY TO CONTEXTUAL FEAR CONDITIONING IN AT1A RECEPTOR KNOCKOUT MICE**Choy K.H.C., Chavez C.A. and Mayorov D.N.  
Dept of Pharmacology, The University of Melbourne.

Our recent studies indicate that angiotensin AT<sub>1A</sub> receptor (AT<sub>1A</sub><sup>-/-</sup>) knockout mice have diminished blood pressure (BP) reactivity to physico-emotional stressors, such as shaker or restraint. It remains uncertain however whether this attenuation is due to reduced emotional reactivity to threatening stimuli, or it reflects a diminished autonomic responsiveness to fearful emotional reactions. Therefore, in this study, we examined the influence of AT<sub>1A</sub> receptors on cardiovascular and behavioural effects of contextual fear conditioning in AT<sub>1A</sub><sup>-/-</sup> (n=6) and AT<sub>1A</sub><sup>+/+</sup> (n=5) mice. Two weeks following implantation of BP telemetry device, mice were pre-exposed to context (the footshock chamber) for three 5-min sessions and then subjected to a 5-min footshock session consisted of 4 brief electric footshocks. Mice were then re-exposed to the same context 4, 24, 48 and 96 hours after the footshock, and their behaviour was analysed by Ethovision video tracking system. Pre-exposure to context similarly increased BP in AT<sub>1A</sub><sup>-/-</sup> and AT<sub>1A</sub><sup>+/+</sup> mice (+29±5 and +31±3 mmHg, respectively). Conversely, the BP rise during the re-exposure sessions was lower in AT<sub>1A</sub><sup>-/-</sup> than AT<sub>1A</sub><sup>+/+</sup> mice (+24±4 and +37±2 mmHg, respectively). However, immobility duration (freezing) during fear conditioning and extinction was similar between groups. Moreover, AT<sub>1A</sub><sup>-/-</sup> mice displayed increased anxiety-like behaviour as evidenced by reduced time spent in the centre of the footshock chamber and also in open arms of the elevated plus maze. These data indicate that AT<sub>1A</sub> receptor deficiency attenuates the pressor response to conditioned contextual fear in mice. This attenuation cannot be ascribed to reduced emotional reactivity or anxiety, and may thus relate principally to dysfunctions in central autonomic regulation.

## POS-MON-112

**EFFECT OF 5HT1A RECEPTOR ACTIVATION IN LOWER BRAINSTEM ON CARDIOVASCULAR AND BEHAVIOURAL RESPONSES TO PSYCHOLOGICAL AND PHYSICAL STRESS**Luong L.N.L., Vianna D.M.L. and Carrive P.  
Wallace Wurth Building, Faculty of Medicine, University of New South Wales, Kensington, Australia 2052.

**Introduction:** In the conscious animal, the activation of the 5HT<sub>1A</sub> receptor via systemic injection of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a 5HT<sub>1A</sub> receptor agonist, has been shown to attenuate the heart rate (HR) and mean arterial pressure (MAP) responses to psychological stress (1). The lower brainstem has been proposed as one site of action (2). Thus, in the anaesthetised animal, intracisternal injection of 8OHDPAT reduces the cardiovascular response to stimulation of the hypothalamic defense area (3). However, it is not known if the same activation of 5HT<sub>1A</sub> receptor in the lower brainstem can also attenuate the cardiovascular and behavioural responses to psychological stress in the conscious animal. **Purpose:** To determine the effect of 5HT<sub>1A</sub> receptor activation in the lower brainstem on cardiovascular and behavioural responses to different types of stressors. **Method:** Animals were implanted with radio-telemetric probes and a cannula aiming at the fourth ventricle. They were microinjected with 10ul of artificial cerebrospinal fluid (aCSF) or 8-OH-DPAT (2ug/kg, 5ug/kg or 10ug/kg) immediately before being exposed to a psychological (Novelty, Restraint or Conditioned Fear) or physiological (Cold Exposure) stressor. **Results:** All four stressors elicited increases in HR and MAP. Administration of 8OHDPAT into the fourth ventricle attenuated HR responses to all four stressors ( $p < 0.01$ ). The MAP responses to Novelty and Conditioned Fear were also significantly reduced ( $p = 0.01$ ); to Restraint, it was close to significance ( $p = 0.06$ ) but to Cold Exposure, it was not significant ( $p = 0.89$ ). 8-OH-DPAT also reduced the locomotor response to Novelty ( $p = 0.02$ ) but not to Cold Exposure ( $p = 0.130$ ). The freezing response to Conditioned Fear was also reduced ( $p = 0.02$ ) but not ultrasonic vocalisations ( $p = 0.66$ ). **Conclusion:** These results, together with our previous work (1) suggest that high systemic doses of 8-OH-DPAT could act on 5HT<sub>1A</sub> receptors in the lower brainstem to reduce the cardiovascular and behavioural responses to psychological stress.

## POS-MON-113

**A MODEL OF ATRIAL PROPAGATION BASED ON *IN VITRO* ACTION POTENTIAL RECORDS**

**Al Abed A.**, Guo T., Dokos S. and Lovell N.H.  
Graduate School of Biomedical Engineering, The University of New South Wales, Sydney, 2052, Australia. s.dokos@unsw.edu.au.

Mathematical models have played an important role in the development of electrophysiology. However there is a need for experiment-specific models. Towards this aim a single-cell ionic model is described, able to reproduce a variety of cardiac action potential (AP) waveforms. The model consists of three ionic currents, two active and one leakage. Each active conductance moves between four states, a process that is controlled by a set of voltage-dependent rates. To test the model's ability to reproduce experimental records, intracellular APs were obtained from *in vitro* rabbit sino-atrial preparations using glass microelectrodes (N=3 cells). Spontaneous APs were recorded from central, peripheral sinus node (SN) and atrial cells. The sinus node APs had a slow depolarisation (pacemaker) phase which was absent in atrial cells. A numerical algorithm was developed to fit the model to a sequence of APs from each cell type. By searching for and using different sets of model parameters, the model is optimised to reproduce the characteristic AP waveforms of central, peripheral SN and atrial cells. The generic nature of the model allows it to be used to simulate electrical activation of heterogeneous tissue. A 3D simulation of atrial electrophysiology is also described using the NIH male Visible Human Dataset atrial geometry with our SN and atrial ionic models assigned to their respective regions. The SN was spontaneously active and able to excite the surrounding atrium replicating normal propagation. The methodology developed in this study allows ionic cell models to be fitted to experimentally recorded data and utilised in anatomically detailed simulations.

## POS-MON-115

**INDIVIDUAL DIFFERENCES IN CARDIOVASCULAR RESPONSE TO FOOTSHOCK BUT NOT SHAKER STRESS PREDICT CONTEXTUAL FEAR CONDITION IN RATS**

**Hawkes D.J.**, Choy K.H.C. and Mayorov D.N.  
Stress Neurobiology Laboratory, Department of Pharmacology, University of Melbourne, Parkville, Vic, 3010.

Excessive cardiovascular reactivity, an abrupt increase in blood pressure (BP) and heart rate (HR), is a risk factor for heart disease. The aim of this study was to examine whether BP and HR reactivity to standardized laboratory stressors, such as shaker stress and footshock, can be used to predict the magnitude of reaction of contextual fear-conditioning in rats. Sprague-Dawley rats (n=4) were implanted with radio-telemetry probes to measure BP, HR and locomotor activity. Two weeks later, animals were pre-exposed to context (footshock chamber) for two 30-min sessions and then to a 30-min footshock session which consisted of 3 electric footshocks (1 mA, 3.5 sec). Animals were re-exposed (post-exposure) to the same context 4 hours after footshock, and their behaviour was analysed using ANY-maze video tracking software. HR and BP were measured pre-, post- and during stress. The increases in BP and HR following shaker stress (60- and 150-rpm) were compared to those seen during footshock and no significant correlations were found between these responses (all  $r^2 < 0.57$ , and all  $p > 0.25$ ). Interestingly, increases in BP during a mild 60-rpm shaker stress were inversely related to those during more severe 150-rpm shaker stress ( $r^2 = 0.91$ ,  $p < 0.05$ ). There was no correlation in BP rises between pre- and post-footshock sessions ( $r^2 = 0.05$ ,  $p < 0.78$ ), but increases during footshock significantly correlated with increases during re-exposure ( $r^2 = 0.965$ ,  $p > 0.018$ ). These data suggest that individual differences in cardiovascular reactivity to acute unconditioned stressor may predict contextual fear condition only if paired with the same context throughout the task. Further molecular studies examining CNS contributors to cardiovascular reactivity to contextual fear are currently in progress.

## POS-MON-114

**HYPERTHERMIA-INDUCED REDUCTION OF MESENTERIC BLOOD FLOW INHIBITED BY NITRIC OXIDE SYNTHASE INHIBITION WITHIN THE PARAVENTRICULAR NUCLEUS IN RATS**

**Chen F.<sup>1</sup>**, Cham J.L.<sup>1</sup>, Wang Y.L.<sup>2</sup> and Badoer E.<sup>1</sup>  
<sup>1</sup>RMIT University, PO BOX 71, Bundoora, Victoria. <sup>2</sup>Department of Physiology, Weifang University, China.

Background: The autonomic reflex response to an altered ambient temperature includes mesenteric vasculature changes. The hypothalamic paraventricular nucleus (PVN) is an important integrative site implicated in hormonal, endocrine, and neural control and may play an essential role in this autonomic reflex. However, the neurochemicals within the PVN mediating the reflex are unknown. Nitric oxide (NO) is involved in temperature regulation and is in high concentration within the PVN. AIM: To determine whether NO in the PVN contributes to the reduction in mesenteric blood flow (MBF) elicited by hyperthermia. Methods: Rats (Sprague-Dawley) were anaesthetised with Equithesin (sodium pentobarbitone (0.5g) + chloral hydrate (2.219g) per 100 ml; 3ml/kg i.p.) followed by maintenance with urethane (0.05g/kg, i.v.) and prepared for monitoring of blood pressure (BP), heart rate (HR) and MBF. Rats were assigned into three groups (n = 5/group) in this study. In first two groups, rats were administered NG-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, bilaterally into the PVN (100nl/side) at a dose of 100 or 200 nmol/100nl. In a third group of rats saline was microinjected into the PVN as a control. Body core temperature of the rats was then elevated to 39°C. Results: In control rats, increasing body core temperature resulted in no marked change of BP but an increase in HR and a significant decrease of MBF (~15%). Pre-treatment with 100 nmol L-NAME did not affect the response. In contrast, 200 nmol L-NAME prevented the normal reduction in MBF but did not affect the BP and HR responses. Conclusion: NO production is increased during hyperthermia in the PVN and may be an important neurochemical in this region to mediate the reduction of MBF induced by hyperthermia.

## POS-MON-116

**DIFFERENT STRESSORS ACTIVATE DIFFERENT PATTERNS OF PREMOTOR SYMPATHETIC GROUPS**

**McConnell A.** and Carrive P.  
Dept of Anatomy, SOMS, UNSW, Kensington NSW 2052, Australia.

It is well established that the vasopressor premotor sympathetic neurons of the rostral ventrolateral medulla (RVLM) are strongly activated during haemorrhage. It has also been proposed that the RVLM is a final common pathway for all sympathetically-mediated pressor responses, including those associated with stress (1). However, recent work shows that conditioned fear, which evokes a marked pressor response, is not associated with RVLM or adrenergic C1 activation but with activation of presympathetic neurons in the perifornical hypothalamus (PeF), paraventricular hypothalamus (Pa) and A5 noradrenergic group (2). The aim of this study was to investigate the presympathetic groups of 2 other stressors, restraint and cold exposure (4°C). Haemorrhage was the positive control and rest the negative control. The retrograde tracer Cholera toxin subunit B (CTB) was injected bilaterally into the upper thoracic cord 2 weeks before test. Brains were then analysed for double immunolabeling of Fos and either CTB or tyrosine hydroxylase. As expected, haemorrhage (n=1) preferentially activated A5 [31.58%], RVLM [24.79%] and RVMM [18.18%] but not PeF [5.7%], Pa [4.69%] or raphe pallidus (RPa) [1.09%]. Restraint (n=3) preferentially activated A5 [37.7%] and Pa [17.7%] but not RVLM [2.90%], RVMM [7.32%], PeF [9.64%] or RPa [10.95%]. Cold exposure (n=3) preferentially activated RPa, [50%], PeF [16.94%] and A5 [17.17%], but not RVLM [5.14%], RVMM [9.26%] or Pa [4.68%]. These early results suggest that the presympathetic neurons of the RVLM contribute very little to the sympathetic response of restraint and cold exposure, unlike haemorrhage. Furthermore, it appears that different patterns of premotor sympathetic activation arise depending on the stressor. 1. Dampney (1994) *Physiol Rev* 74, 323-64 2. Carrive and Gorissen (2008) *EJN* 28, 428-46.

## POS-MON-117

## THE EFFECTS OF HEMORRHAGIC SHOCK AND RESUSCITATION ON INTRA-ABDOMINAL PRESSURE

Yoshino O.<sup>1</sup>, Quail A.<sup>2</sup> and Balogh Z.<sup>1</sup><sup>1</sup>Department of Traumatology, John Hunter Hospital and University of Newcastle. <sup>2</sup>Discipline of Human Physiology, School of Biomedical Science and Pharmacy, Faculty of Health, University of Newcastle.

Elevated intra-abdominal pressure (IAP) produces detrimental effects on abdominal organs in abdominal compartment syndrome (ACS). It is postulated that severe ischemia and reperfusion injury may be the main cause of increased IAP and ACS. The aims were to quantitate the effect of haemorrhagic shock, resuscitation (including timing of blood transfusion) on IAP. Three groups of 6 rabbits were anaesthetised (isoflurane) and instrumented with central venous, peritoneal and arterial catheters. Arterial blood gases, blood pressure, heart rate, central venous pressure and IAP were monitored. Group 1 served as a sham without haemorrhagic shock. Group 2 and 3 were bled to induce haemorrhagic shock (mean arterial pressure maintained at ~30 mmHg for 1 hour), followed by resuscitation over 5 hours with Lactated Ringer and early (immediate) return of shed blood in Group 2; and with Lactated Ringer and delayed (after 180 minutes) return of shed blood in Group 3. Physiological parameters were unchanged in the sham group, while Group 2 and 3 were successfully resuscitated following severe haemorrhagic shock based on vital signs and blood gases. IAP in Group 1 was stable at  $0.9 \pm 0.16$  mmHg (mean  $\pm$  SE), whereas Group 2 and 3 had significant increases in IAP to  $3.1 \pm 0.38$  mmHg ( $P < 0.05$ ) and  $3.8 \pm 0.34$  mmHg ( $P < 0.05$ ) respectively at 5 hours. IAP increased significantly after 240 and 150 min in Group 2 and 3 respectively. It is concluded that haemorrhagic shock and subsequent resuscitation increased IAP in the rabbit to a maximum 4 mmHg. Early resuscitation with blood transfusion potentially alleviated the effect of haemorrhagic shock on IAP.

## POS-MON-118

## EVIDENCE FOR A GABAERGIC CONNECTION BETWEEN THE CENTRAL NUCLEUS OF THE AMYGDALA AND THE PERIAQUEDUCTAL GRAY IN THE MOUSE

Shivapathasundram G.<sup>1</sup>, Chieng B.C.<sup>2</sup> and Carrive P.<sup>1</sup><sup>1</sup>School of Medical Sciences, University of New South Wales. <sup>2</sup>Brain and Mind Research Institute, University of Sydney.

Conditioned fear elicits a series of responses that are thought to be mediated by the central nucleus of the amygdala (CeA). One such response, freezing immobility, is mediated by a direct connection between the CeA and the ventrolateral periaqueductal gray (VLPAG). There is growing evidence that the output of the CeA is GABAergic and inhibitory. **Purpose:** We sought to investigate the nature of the connection between the CeA and VLPAG using glutamic acid decarboxylase 67-green fluorescent protein (GAD67-GFP) knock-in transgenic mice. **Methods:** GAD67-GFP knock-in transgenic mice ( $n=6$ ), in which GABAergic neurons express GFP received injections of the retrograde tracer Cholera Toxin subunit b (CTb) in the VLPAG. Double-immunolabelled GFP-CTb and single immunolabelled CTb neurons were plotted and counted throughout the amygdala. **Results:** The CeA had the densest distribution of both single-labelled (CTb)(57%) and double-labelled (GFP-CTb) cells (40%). The second largest distribution of double-labelling was in medial nucleus and the basomedial complex of the amygdala (18%). **Conclusion:** A significant proportion (40%) of CeA output neurons projecting to the VLPAG in the mouse are GABAergic. This proportion is less than in the rat as revealed by in situ hybridization for GAD67 (93% [1] and 66% [2]). It is not clear if this difference is species specific or due to different sensitivities of the techniques. Nevertheless, it raises important questions regarding the mechanisms of activation of the VLPAG during conditioned fear. 1. Olsen et al, (2009) Proc Austr Neurosci POS-WED-185. 2. Oka et al, (2008) Neurosci Res 62: 286-298.

## POS-MON-119

## PHYSIOLOGICAL PROPERTIES OF THE PARABRACHIAL-CENTRAL AMYGDALOID SYNAPSE IN A RAT MODEL OF NEUROPATHIC PAIN

Denny J.C.<sup>1</sup>, Crane J.W.<sup>2</sup> and Delaney A.J.<sup>2,1</sup><sup>1</sup>School of Biomedical Science, The University of Queensland, QLD 4072 Australia. <sup>2</sup>The Queensland Brain Institute, The University of Queensland, QLD 4072 Australia.

Pain is a multidimensional experience. While the sensory/discriminatory aspect of pain is well understood, the emotional aspect remains mostly unexplored. It is thought that nociceptive information is relayed to the CNS for emotional processing by the ascending spino-parabrachial-amygdaloid pathway. Here, we examined the physiology of parabrachial (PB) perisomatic basket terminals synapsing onto lateral central amygdala (CeAL) neurons in experimental autoimmune neuritis (EAN). In response to stimulation of the PB fibres, CeAL neurons displayed large all-or-none EPSCs that were inhibited by NAd. We observed no change in EPSC amplitude (EAN:  $-115.2 \pm 15.43$  pA,  $n = 16$  cells from 7 animals) (Control:  $-101.3 \pm 13.98$  pA,  $n = 14$  cells from 6 animals), paired pulse ratio (EAN:  $1.402 \pm 0.061$ ,  $n = 16$  cells from 7 animals) (Control:  $1.395 \pm 0.082$ ,  $n = 14$  cells from 6 animals), or percentage inhibition by an EC<sub>50</sub> concentration of NAd (EAN:  $44.15 \pm 11.76\%$ ,  $n = 7$  cells from 3 animals) (Control:  $36.54 \pm 11.90\%$ ,  $n = 5$  cells from 3 animals). Additionally, basal numbers of Fos-positive nuclei within the CeA did not differ between EAN and Control animals (EAN:  $239.5 \pm 54.78$ ,  $n = 10$ ) (Control:  $236.8 \pm 40.57$ ,  $n = 10$ ). Our results suggest the PB-CeAL synapse does not undergo synaptic plasticity or neuromodulatory changes during EAN. This may be due to compensatory mechanisms, or be an indication of the importance of this synapse in the emotional processing of pain.

## POS-MON-120

## EFFECT OF PRAESCENT™ ON STRESS-INDUCED CHANGES IN THE BASOLATERAL AMYGDALA

Mu E., Spiers J.G., Noakes P.G. and Lavidis N.A.

Synaptic Biology Group, School of Biomedical Sciences, Faculty of Science, The University of Queensland, Brisbane, Queensland, Australia.

Numerous studies have shown that the basolateral amygdala (BLA) is central to stress regulation, and that chronic stress can lead to detrimental morphological changes. These may be responsible for post-traumatic stress disorder (PTSD), anxiety disorders and depression. Studies have also shown a direct anatomical link between the olfactory pathway and the amygdala, suggesting that olfaction can mediate stress responses. **Purpose:** This study aimed to determine whether Praescent™ (*cis*-3-hexen-1-ol, *trans*-2-hexenal and  $\alpha$ -pinene) could attenuate stress-induced morphological changes in the pyramidal neurons of the BLA. **Methods:** Male Wistar rats ( $n=24$ ) were exposed to different treatments (control, vehicle only, Praescent™ only, stress only, stress and vehicle, and stress and Praescent™) for 4 hours over 21 consecutive days. Neuronal cell counts and BLA volume were determined in Nissl-stained brain slices using neurostereological software. Golgi-impregnated dendrites were analysed with Image J software to estimate apical dendritic length and dendritic branching patterns. **Results:** Rats exposed to stress only and stress with vehicle treatments experienced an approximate  $38.81 \pm 4.47\%$  and  $41.32 \pm 2.20\%$  increase in pyramidal neurons compared to the control group ( $P < 0.05$ ), while stress and Praescent™ treated rats had similar neuron counts to the control. These rats also demonstrated a  $26.13 \pm 4.76\%$  and a  $27.09 \pm 1.84\%$  increase in apical dendritic length ( $P < 0.05$ ) and increased branching (65 - 80  $\mu$ m from soma) compared to the control (30 - 50  $\mu$ m from soma) ( $P < 0.05$ ). **Conclusion:** These findings show that Praescent™ can significantly reduce stress-induced morphological changes in the BLA. This further suggests that Praescent™ could potentially prevent the onset of PTSD and other psychiatric disorders caused by dysfunctional amygdala activity during stress.



## POS-MON-121

**EVIDENCE OF A GABAERGIC PROJECTION FROM THE CENTRAL NUCLEUS OF THE AMYGDALA TO THE VENTROLATERAL PERIAQUEDUCTAL GRAY**Olsen N.D.<sup>1</sup>, Kumar N.N.<sup>2</sup>, Goodchild A.K.<sup>2</sup> and Carrive P.<sup>1</sup><sup>1</sup>School of Medical Sciences, University of New South Wales. <sup>2</sup>The Australian School of Advanced Medicine, Macquarie University.

It is emerging that neurons in the central nucleus of the amygdala are GABAergic rather than glutamatergic. However, it is not clear if this is also true for output neurons to the brainstem and in particular to the ventrolateral periaqueductal gray (VLPAG). Purpose: To determine the relative distribution of GABAergic and glutamatergic projections from the amygdala to the VLPAG. Methods: The retrograde tracer cholera toxin subunit B (CTB) was injected into the caudal VLPAG of rats, and *in situ* hybridisation was used to reveal glutamic acid decarboxylase 67 mRNA (GAD67; n = 6) and vesicular glutamate transporter 2 mRNA (VGLUT2; n = 3). Single and double labelled cells were counted throughout the amygdala. Results: Retrogradely labelled cells were found mainly in the medial part of the central nucleus (CeM) (approximately 44% of all CTB immunoreactive cells in the amygdala), followed by the medial amygdala (MeA; 27%), basomedial amygdala (BMA; 9%) and the lateral and capsular parts of the central nucleus (CeL and CeC; 7% each). The proportion of CTB immunoreactive neurons double-labelled with GAD67 and VGLUT2 was, respectively, 93% and 0% in CeM; 93% and 0% in CeL; 88% and 0% in CeC; 52% and 57% in the dorsal MeA; 14% and 78% in the ventral MeA; and 22% and 63% in the BMA. Conclusion: The central nucleus is the main output nucleus of the amygdala to the VLPAG and is almost exclusively GABAergic. MeA and BMA contain glutamatergic VLPAG-projecting neurons, but these represent a minor proportion of the total amygdala output to the VLPAG.

## POS-MON-123

**TOPOGRAPHICAL SPECIFICITY OF RESPIRATORY REGULATION BY THE DORSOLATERAL PERIAQUEDUCTAL GREY**Ilgaya K., Horiuchi J., McDowall L.M. and Dampney R.A.L.  
School of Medical Sciences (Physiology) and Bosch Institute,  
University of Sydney, NSW 2006, Australia.

Previous studies have reported that the neurons in the dorsal and lateral parts of the midbrain periaqueductal grey (PAG) can exert strong effects on sympathetic activity and respiration (1,2). The PAG subregions (dorsomedial, dorsolateral and lateral) differ greatly with respect to their anatomical connections. In the present study, we tested whether there are also differences with respect to their functional control of sympathetic and respiratory activity. Arterial pressure, heart rate, renal sympathetic nerve activity (RSNA) and phrenic nerve activity (PNA) were recorded in rats (n=11) anaesthetized with urethane. Microinjections of D,L-homocysteic acid (750 pmol) evoked large increases in PNA burst rate and amplitude ( $50 \pm 12$  and  $42 \pm 13\%$ , respectively) from sites within the dorsolateral PAG at the level 7.6 mm caudal to bregma, but much smaller effects ( $P < 0.01$ ) from sites in the surrounding PAG subregions. The respiratory effects evoked from the dorsolateral PAG were also accompanied by a large increase in RSNA ( $39 \pm 10\%$ ), but in contrast to the respiratory effects large sympathetic responses were also evoked from the dorsomedial and lateral PAG. The results indicate that cells within a circumscribed region in the dorsolateral PAG has a strong effect on respiratory activity, which could be mediated via ascending projections to the dorsomedial hypothalamus (1). 1) Horiuchi J et al., J Physiol 587: 5149-5162, 2009. 2) Subramaniam HH et al., J Neurosci 28: 12274-12283, 2008.

## POS-MON-122

**CATECHOLAMINE NEUROTRANSMISSION IN THE ORBITAL FRONTAL CORTEX EVOKED BY STIMULATION OF THE VENTRAL TEGMENTAL AREA**Tye S.J.<sup>1,2</sup>, Covey D.P.<sup>3</sup>, Griessenauer C.J.<sup>4</sup>, Garriss P.A.<sup>3</sup> and Lee K.H.<sup>1,5</sup><sup>1</sup>Dept. Neurosurgery, Mayo Clinic, Rochester MN USA. <sup>2</sup>Dept. Psychology, Deakin University, Burwood VIC Australia. <sup>3</sup>Dept. Biological Sciences, Illinois State University, Normal IL USA. <sup>4</sup>Dept. Surgery, University of Alabama, Birmingham AL USA. <sup>5</sup>Dept. Physiology and Biomedical Engineering, Mayo Clinic, Rochester MN USA.

The orbital frontal cortex (OFC) and ventral tegmental area (VTA) are critically involved in processing information about the relative value of reinforcers and cues predicting reward, and are essential for learning from unexpected outcomes. Each region is mediated by stimulation of the nucleus accumbens (NAc) and this pathway may thus form an important network that is modulated by NAc deep brain stimulation (DBS). To assess the functional connectivity of these regions we have utilised the Wireless Instantaneous Neurotransmitter Concentration System (WINCS) in fast scan cyclic voltammetry (FSCV) mode, coupled with a carbon fibre microelectrode (CFM), to monitor VTA-evoked (300-350  $\mu$ A, 60 Hz) catecholamine release in medial regions of the OFC (0.5 mm lateral to midline) of urethane anaesthetised male rats *in vivo*. VTA stimulation was optimised first by measuring forebrain dopamine release in the NAc. Once established, a CFM was placed in the OFC and VTA-evoked voltammetric currents monitored. VTA-evoked OFC signals were maximal in the upper portion of the medial OFC (MO) 3.5 mm ventral from skull surface. Smaller catecholamine responses were, however, also observed in the lower portions of the prelimbic and ventral orbital regions of the frontal cortex along this trajectory. The maximal VTA-evoked catecholamine signal in the MO was responsive to both noradrenergic and dopaminergic pharmacological manipulations, suggestive of a highly integrated dopaminergic/noradrenergic response. These VTA-mediated NAc dopaminergic and OFC catecholaminergic responses are likely to have important consequences for interrelated mood, obsessive-compulsive and addictive disorders, and represent a potential network disrupted by NAc DBS. FSCV evaluation of DBS mechanisms for psychiatric indications has the potential to proffer important new insight into the mechanism of action of this new and evolving neuromodulation therapy.

## POS-MON-124

**INTENSITY-DEPENDENT BRAIN RESPONSES DURING CAPSAICIN INHALATION**Farrell M.J.<sup>1,2</sup>, Cole L.J.<sup>1</sup>, Chiapoco D.<sup>2</sup> and Mazzone S.<sup>3</sup><sup>1</sup>Florey Neurosciences Institutes. <sup>2</sup>Centre for Neuroscience, University of Melbourne. <sup>3</sup>Biomedical Sciences, University of Queensland.

**PURPOSE:** Inhalations of increasing concentrations of capsaicin solution are associated with increasing ratings of urge-to-cough and increasing likelihood of cough. A widely distributed pattern of brain activation is associated with inhalation of high doses of capsaicin that represents sensory and motor responses during airways irritation and cough suppression. We hypothesised that graduated urge-to-cough and cough suppression during inhalation of capsaicin would be associated with increased brain activation at higher compared to lower concentrations of capsaicin. **METHODS:** Functional brain images using blood oxygen level-dependent (BOLD) contrast were acquired with a Siemens 3T scanner from healthy volunteers (n=13) during 42s blocks of rest interleaved with 18s blocks of inhalation via nebuliser of either saline, or a low concentration or high concentration of capsaicin relative to each individual's capsaicin cough threshold. General linear modeling was used to identify variance in BOLD signals associated with inhalation events, and contrasts were generated to identify capsaicin intensity-dependent activation. **RESULTS:** Capsaicin inhalation was associated with increased BOLD signal intensity in distributed brain regions including SI, M1, the insula, cingulate, and posterior parietal cortices. Almost all regions of activation showed significantly greater levels of BOLD signal intensity for high versus low concentrations of capsaicin ( $p_{corrected} < 0.05$ ) with the exception of a cluster in the right inferior parietal lobule (IPL, BA40). **CONCLUSIONS:** Intensity-dependent changes in brain responses during inhalation of capsaicin are consistent with sensory experiences and the relative risk of coughing that was suppressed during the experiment. Activation in the IPL could represent attention processes involving integration of sensory inputs, which is a function that has been ascribed to this region, and which is unlikely to be dependent on sensory intensity.

## POS-MON-125

## HYPOTHALAMIC REGIONS ACTIVATED DURING VOLUNTARY EXERCISE AND AIRPUFF STARTLE

Lam A.C.B., Horiuchi J., McDowall L.M., Furlong T., Iigaya K., Polson J.W. and Dampney R.A.L.  
School of Medical Sciences (Physiology) and Bosch Institute, The University of Sydney, NSW 2006, Australia.

Both exercise and acute psychological stress are associated with stereotyped cardiovascular and respiratory responses. The hypothalamus is believed to play a key role in generating these responses. In this study we used the method of Fos expression to compare the pattern of neuronal activation in the hypothalamus of rats after a period of either voluntary exercise or a mild acute psychological stress (air puff startle). In the exercise group, rats (n=5) had free access to a running wheel. Two hours after the period in which the running activity was maximal (4-6 am), rats were euthanized with an overdose of sodium pentobarbitone, and the brains removed and processed to identify Fos-positive neurons. Exercise control rats (n=4) were housed without the running wheel and euthanized at the same time of day as the exercising rats. Another group of rats (n=4) were subjected to air puff startle and euthanized 2 hours later. After both airpuff and voluntary exercise, there was a marked increase in Fos expression in the hypothalamus, but the patterns were quite different. After voluntary exercise there was much greater activation in the hypothalamic perifornical area compared with the dorsomedial hypothalamus (DMH) ( $P < 0.05$ ), whereas after airpuff startle the reverse was the case ( $P < 0.05$ ). A high proportion ( $> 60\%$ ) of orexin-containing neurons in the perifornical area expressed Fos after voluntary exercise. The results suggest that the central mechanisms generating cardiorespiratory responses to stress and exercise may, at least at the level of the hypothalamus, be quite different.

## POS-MON-126

## THE ROLE OF NEOGENIN AND ITS LIGAND, RGMa, IN DIFFERENTIATION IN THE ADULT BRAIN

Bradford D. and Cooper H.M.  
Queensland Brain Institute, Building 79, UQ St Lucia, QLD 4072.

**Purpose:** The ability of the adult brain to produce neurons is well established. The molecular mechanisms controlling differentiation to a neural fate and a specific neuronal subtype are still poorly understood. The largest proliferative region in the adult brain is the subventricular zone (SVZ) where cells are initially quiescent and then differentiate into neuronal precursors. New neurons migrate along the rostral migratory stream to the olfactory bulb (OB) where they integrate into the granule cell and glomerular layers. The multi-functional receptor, Neogenin, is expressed in the SVZ, and one of its ligands, RGMa, is expressed in a complementary pattern. Our studies indicate these molecules have a role in differentiation leading to production of a specific subset of neurons. **Method:** In vitro functional studies were conducted on neurospheres generated from Neogenin gene trap and wild-type mice (n=5). In vivo analysis was performed in Neogenin gene trap and wild-type mice (n=5). **Results:** Our experiments show differentiated neurospheres from Neogenin gene trap mice have significantly fewer neurons than wild-type mice; and RGMa appears to regulate differentiation. We find that this effect is limited to a specific subset of interneurons. Our in vivo analysis comparing neogenin gene trap mice with their wildtype littermates suggests that the ramifications of this effect are seen in the granule cell layer of the OB. Further comparisons between these genotypes show a corresponding effect in the granule cell layer of the cerebellum. **Conclusion:** Together, these data suggest Neogenin and RGMa have a role in differentiation, not just to a neural fate, but also to a specific subtype of interneuron. Our preliminary human studies show that Neogenin and RGMa are present in the human SVZ possibly indicating that their role in differentiation is conserved in humans.

## POS-MON-127

## THE FOVEA AND AREA DORSALIS DEVELOP INDEPENDENTLY IN THE PIGEON (COLUMBA LIVIA) RETINA

Bumsted O'Brien K. and Hickey D.  
ARC Centre for Excellence in Vision Science, Australian National University.

**PURPOSE:** The pigeon retina has two high acuity regions, the fovea and the area dorsalis in the red field. There is little information concerning the morphological development of these regions. The aim of this study was to analyse the formation fovea and area dorsalis during retinal development. **METHOD:** A series of eyes from post-hatch (P) pigeons (n=34) (*Columba livia*) were fixed in 4% paraformaldehyde. Flatmounted retinas were used for photoreceptor and ganglion cell density counts and rod opsin immunocytochemistry. Frozen sections were stained with Cresyl violet for morphological analysis. **RESULTS:** The incipient fovea at P0 was characterized by cones forming a single layer of cuboidal cells and doming of the ganglion cells at the fovea. The pigeon fovea was detected 2.3mm nasal to the optic disc at P7 days. As development proceeded, cone density increased in the fovea and the ganglion cell density decreased. Ganglion cells moved laterally to form a pit. The area dorsalis contained a higher density of photoreceptors at hatching compared with the fovea, and this density slowly increased until adulthood. **CONCLUSION:** These data demonstrate that pigeon fovea develops posthatch and follows a morphological progression similar to primate foveal formation. The area dorsalis develops high photoreceptor densities pre-hatch and develops at a different temporal rate compared with the fovea. Therefore, the pigeon fovea is a good model for primate foveal development in that it shares a number of important morphological and functional characteristics. The area dorsalis will provide a useful comparison to the fovea in that the development of two independent high acuity regions can be studied.

## POS-MON-128

## METHODOLOGIES FOR THE SPECIFIC ISOLATION OF MIDBRAIN DOPAMINE NEURONS FOR GENE AND PROTEIN EXPRESSION PROFILING

Bye C.R., Blakely B.D., Fernando C.V., Horne M.K., Thompson L.H. and Parish C.L.  
Brain Injury and Repair, Florey Neuroscience Institutes, University of Melbourne, Australia.

The heterogeneity of neuronal nuclei in the brain complicates any attempt to profile gene or protein expression from specific neuronal populations. The generation of a sensitive assay of expression changes necessitates the specific isolation of the population of interest, otherwise the profile generated will become diluted and confounded by the presence of unrelated cell populations. A specific isolation becomes essential when the nuclei of interest is small and the contribution of the unrelated cell populations outweighs that of the target population. This is true for the dopaminergic neurons of the ventral midbrain. In this proof of principle study the ventral midbrain dissected from TH-GFP mice (without the specific isolation of dopamine neurons) was compared to laser capture microdissected SNpc cells as well as dissociated and sorted (fluorescently or magnetically) dopamine neurons. Non-specifically isolated midbrain, even in the presence of GFP to guide the dissection, produced an average of 5% dopamine neurons as a percentage of the dissected cells. The feasibility of the LCM and sorting methodologies were demonstrated, both yielding mRNA and protein of high quality and of a sufficient quantity for application to commonly used expression assays. A comparison of the expression profiles between the specific and non-specifically isolated cell populations revealed a vast increase in the number and magnitude of the expression changes detected in the specifically isolated populations. This study showed the specific isolation of the dopaminergic population is required for the sensitive detection of expression changes from these neurons. The limitation and feasibility of the isolation methodologies are discussed in the context of every day research.

## POS-MON-129

## THE ROLE OF TYROSINE HYDROXYLASE DURING EARLY DOPAMINE DEVELOPMENT IN THE ZEBRAFISH EMBRYO (DANIO RERIO)

**Formella I.<sup>1</sup>, Scott E.K.<sup>1,2</sup>, McGrath J.J.<sup>1,3</sup>, Burne T.H.J.<sup>1,3</sup> and Eyles D.W.<sup>1,3</sup>**  
<sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia. <sup>2</sup>School of Biomedical Science, University of Queensland, St Lucia, QLD 4072 Australia. <sup>3</sup>Queensland Center for Mental Health Research, Wacol, QLD 4076 Australia.

**Purpose:** Tyrosine hydroxylase (*th*) is the rate-limiting enzyme in the biosynthetic pathway of the catecholamine neurotransmitter dopamine and its derivatives. *Tyrosine hydroxylase* is expressed in all dopaminergic neurons (DA). A variety of neuropsychiatric diseases, such as schizophrenia, are associated with a specific dysfunction of DA neurons in the brain. Our group is pursuing the idea that adverse developmental events that are risk factors for schizophrenia i.e. maternal infection, obstetric complications and low maternal vitamin D, alter the development of DA neurons. A model that interferes with DA ontogeny may prove to be informative in understanding how disorders with a developmental basis such as schizophrenia also show alterations in adult DA signaling. **Method:** Our approach has been to target the *th* gene using *th* splice-site Morpholino oligonucleotides (MO*th*) and *in vitro* synthesized *th* mRNA injection methods in zebrafish embryos. This allows us both to inhibit *th* translation as well as induce ectopic expression of *th* ectopically respectively. **Results:** Preliminary data indicate that 48% of MO*th* injected zebrafish embryos (n=54) show a reduced level of *th* expression at 24 hours post-fertilization. **Conclusion:** Our aim now is to examine the downstream consequences of altered *th* expression such as changes in DA synthesis, DA neuron connectivity and ultimately DA-mediated behaviours in adult zebrafish. The zebrafish represents a model organism with multiple advantages over traditional rodent based models in terms of genetic manipulation, transparent access to the brain during development and rapid developmental time frames for intervention.

## POS-MON-131

## ZEBRAFISH NICAISTRIN IS REQUIRED FOR MID AND HINDBRAIN DEVELOPMENT

**Chong S.W.<sup>1</sup>, Amsterdam A.<sup>2</sup>, Ang H.S.<sup>3</sup>, Yang H.<sup>3</sup>, Hopkins N.<sup>2</sup> and Jiang Y.J.<sup>1</sup>**

<sup>1</sup>Institute of Molecular and Cell Biology, Singapore. <sup>2</sup>Massachusetts Institute of Technology, U.S.A. <sup>3</sup>Singapore immunology Network, Singapore.

Nicastrin (Ncstn), a transmembrane glycoprotein, is an integral component of the gamma-secretase complex that is responsible for cleaving the beta-amyloid precursor protein to produce amyloid beta and the intracellular domain. Ncstn has thus been implicated in Alzheimer's disease (AD). Currently, there is no report on the role of Ncstn in the embryonic development of zebrafish. We sought to validate that mutant *hi1384* from previous retroviral insertion screen is due to a mutation in the zebrafish *ncstn* and to analyze its mutant phenotype. To validate this, we demonstrated that morpholino (MO) against *ncstn* phenocopies *hi1384*<sup>-/-</sup> (n=217, 100%). Data from RT-PCR supported our MO and mutant analyses. Furthermore, we were able to partially rescue its phenotype (n=181, 73%) using the full-length *ncstn* mRNA. These data confirm that *hi1384*<sup>-/-</sup> is deficient in *ncstn*. Notably, our microarray data between zebrafish *ncstn*<sup>hi1384</sup> mutant and wild-type showed highly dynamic transcriptional profile of genes associated with studies in Alzheimer's disease. Some of these genes include *aebp2*, *apoa4*, *casp3*, *ctsl* and *gfap*. Since *caspase 3* is one of the down-regulated genes, we examined related genes such as *acinus* and found that Ncstn is required for neuronal development in the midbrain and hindbrain of zebrafish as shown by using the *ncstn*<sup>hi1384</sup>-Tg(*acinus*:GFP) line (n=137, 100%). The results of these analyses indicate that zebrafish *ncstn*<sup>hi1384</sup> could potentially be used as an animal model for the study of Alzheimer's disease.

## POS-MON-130

## MOLECULAR CHARACTERISATION OF TARGETED PROLIFERATIVE CELLS

**Cavanagh B.<sup>1</sup>, Poulson S.<sup>2</sup>, Jesuadian S.<sup>1</sup>, Dwyer P.<sup>1</sup>, Nguyen M.<sup>1</sup>, Bellette B.<sup>1</sup>, Karunaratne A.<sup>1</sup>, Vaswani K.<sup>1</sup>, Mackay-Sim A.<sup>1</sup> and Meedeniya A.C.B.<sup>1</sup>**

<sup>1</sup>National Centre for Adult Stem Cell Research, ESKITIS Institute, Griffith University, Nathan, Australia. <sup>2</sup>Biological Chemistry, Griffith University, Nathan, Australia.

Proliferating cells labelled using the thymidine analogue BrdU may be characterised using several techniques including *in situ* hybridisation and immunohistochemistry. The extraction of molecular information from these cells has however been unviable due to the high temperatures needed to detect BrdU. We recently demonstrated the efficient detection of proliferating cells *in vivo* and *in vitro* using a novel thymidine analogue EdU, whose detection is dependant on click chemistry. We now demonstrate the efficient extraction of mRNA from proliferating cells, *in vivo* & *in vitro* from cells isolated following EdU incorporation. The olfactory epithelium of young mice was harvested one day (n=46) or seven days (n=48) post EdU exposure (100 mg/kg, i.p.). Mouse embryonic stem cells were exposed to a 10mM EdU for 4 hours prior to harvest. The cells were dissociated to a single cell suspension, fluorescently labelled using Click chemistry and the EdU positive population isolated using fluorescence activated cell sorting (FACS). Molecular profiling of the cells at each stage of the experiment allowed RNA viability to be ascertained. The average RNA yield from embryonic stem cell cultures (n=12) was 1µg/µl for 1 million cells with an absorbance ratio for A260/280, of 2.0. The average amount of RNA extracted from the olfactory epithelium was 0.1µg/µl, with an absorbance ratio of 2.0. We demonstrate the efficient extraction of mRNA for molecular profiling of proliferating cells using EdU in combination with FACS. We obtain yields suitable for microarray analysis and real time PCR for quantitative assay of gene expression profiles.

## POS-MON-132

## ROLE OF INSULIN-REGULATED AMINOPEPTIDASE (IRAP) IN EMBRYONIC NEUROGENESIS

**Diwakarla S., Burns P., Albiston A. and Chai S.Y.**

Florey Neuroscience Institutes, University of Melbourne, Parkville, 3010, VIC, Australia.

Neurogenesis, the generation of new neurons, occurs during embryonic and fetal development and, to a lesser extent, in adults. One of the main areas where neurogenesis occurs in the central nervous system (CNS) is the subventricular zone, where newly produced cells migrate to form the layers of the cerebral cortex and hippocampus. Insulin-regulated aminopeptidase (IRAP) is a metallopeptidase that is highly expressed in pyramidal neurons in the cortex and hippocampus. Inhibition of IRAP activity results in enhanced performance in a number of memory tasks. We recently found high IRAP expression in the subventricular zone of embryonic mouse brain, a brain region that is highly neurogenic. In this study, we investigated the involvement of IRAP in neurogenesis and brain development using the global IRAP knockout mice. Wildtype and IRAP knockout mice (n=7) were injected with 50 mg/kg Bromodeoxyuridine (BrdU), a thymidine analog that labels proliferating cells, and killed after 2h. Embryos were collected and 20 µm cryostat sections cut. Double immunolabelling for BrdU and IRAP was performed and the number of positively stained cells in the subventricular zone was quantified. In addition, CNS development was monitored using Nissl staining followed by measurement of cortical layer thickness. Interestingly, IRAP knockout mice exhibited fewer BrdU-positive cells compared to wildtype mice and a reduction in cortical layer development. These results indicate that IRAP may play a role in neuron production and cell migration. Although IRAP knockout mice show no neurological deficits in adulthood, these results indicate that IRAP may play a role in neuronal development, differentiation and migration.



## POS-MON-133

### THE OLIGODENDROCYTE SPECIFIC TRANSCRIPTIONAL REGULATOR MRF IS VITAL FOR OLIGODENDROCYTE DEVELOPMENT AND CNS MYELINATION

Emery B.<sup>1</sup>, Agalliu D.<sup>2</sup>, Watkins T.<sup>2</sup>, Cahoy J.<sup>2</sup>, Mulinyawe S.<sup>2</sup> and Barres B.A.<sup>2</sup>

<sup>1</sup>Centre for Neuroscience, University of Melbourne, Vic. <sup>2</sup>Department of Neurobiology, Stanford University, CA.

The regulation of oligodendrocyte specification, differentiation and myelination is highly complex and requires the coordinated action of a large number of transcription factors, including Nkx, Sox and Olig family members. Interestingly, the majority of the factors that have been previously identified as being important for oligodendrocyte development are either present at all stages of the oligodendrocyte lineage, or, in the case of Nkx6-2, expressed only in postmitotic oligodendrocytes but not necessary for most aspects of myelination. This contrasts with myelination in the PNS, where the transcription factor Krox20 has been demonstrated to be both specific to and necessary for the generation of myelinating Schwann cells. We have recently identified a novel oligodendrocyte transcriptional regulator, Myelin-gene Regulatory Factor (MRF). Within the CNS, MRF is specifically expressed by postmitotic oligodendrocytes. MRF is a nuclear protein containing an evolutionarily conserved DNA binding domain homologous to a yeast transcription factor. Knockdown of MRF in cultured oligodendrocytes by RNA interference prevents expression of most CNS myelin genes; conversely, forced expression of MRF within cultured oligodendrocyte progenitors or the developing chick spinal cord induces expression of myelin genes. In mice lacking MRF within the oligodendrocyte lineage postmitotic oligodendrocytes are generated but display severe deficits in myelin gene expression and fail to myelinate, largely undergoing apoptosis instead. These mice die due to seizures during the third postnatal week. These findings establish MRF as a critical transcriptional regulator essential for oligodendrocyte maturation and CNS myelination.

## POS-MON-135

### ANALYSIS OF THE ROLE OF OLIGODENDROCYTE EXPRESSED TRKB IN OLIGODENDROCYTE MYELINATION

Wong A.W.<sup>1</sup>, Xiao J.<sup>1</sup>, Kemper D.<sup>2</sup>, Kilpatrick T.J.<sup>1,2</sup> and Murray S.S.<sup>1,2</sup>

<sup>1</sup>Centre for Neuroscience, The University of Melbourne. <sup>2</sup>Florey Neuroscience Institutes.

In the Central Nervous System, myelination is achieved by oligodendrocytes, which extend a multi-lamellar membrane sheath around axons, that express a number of unique glycoproteins collectively known as myelin. The mechanisms required to achieve this process is yet to be fully elucidated. Our laboratory has examined the role of Brain-Derived Neurotrophic Factor (BDNF) in oligodendrocyte myelination, and found that, using *in vitro* myelination assays, BDNF promoted oligodendrocyte myelination, and that this was achieved by direct stimulation of the tyrosine kinase receptor TrkB expressed on oligodendrocytes. To verify these findings *in vivo*, we have generated mice with oligodendrocyte specific deletion of TrkB (TrkB<sup>fl/fl</sup> MBP cre<sup>+</sup> mice). Analyses of these mice at P30 indicate that the TrkB conditional knockout mice exhibit a reduction in expression of myelin basic protein (MBP) in the spinal cord, cerebrum and cerebellum, compared to wild type littermates (n=4). In addition, the expression of another myelin protein, myelin oligodendrocyte protein (MOG), is also reduced in the cerebellum (n=4). Interestingly, immunohistochemical analyses of P30 spinal cord has shown no difference in the number of mature (CC1+) oligodendrocytes between knockouts and controls; however, we do observe a significant increase in the number of (NG2+) oligodendrocyte progenitor cells in the ventral horn of TrkB conditional knockout mice (n=6). We conclude deletion of TrkB results in the reduction of myelin proteins and an endogenous proliferative response amongst oligodendrocyte progenitors, suggesting a compensatory mechanism in this animal model. We are currently exploring the consequence of these events in aging, and will extend our studies to how remyelination is affected in the TrkB conditional knockout mice.

## POS-MON-134

### OLIGODENDROCYTE LINEAGE ELABORATION IN HUMAN FETAL SPINAL CORD DERIVED NEURAL PRECURSOR CELLS

Lovelace M.D.<sup>1</sup>, Weible M.W.<sup>2</sup> and Chan-Ling T.<sup>1</sup>

<sup>1</sup>Discipline of Anatomy and Histology and Bosch Institute, The University of Sydney, NSW, 2006. <sup>2</sup>Eskitis Institute for Cell and Molecular Therapies, Griffith University, Qld, 4111.

**Introduction** - Determining the *in vitro* conditions to derive and maintain oligodendrocytic precursor cells (OPCs) from human fetal spinal cord is a vital step in the development of cell therapies for the treatment of demyelinating disease. This work aimed to characterise the development, expansion of, and functional incorporation of OPCs in animal transplant models. **Methods** - Human fetal 13-19 week spinal cords (n=9) were expanded in neurobasal media with EGF/FGF. 14 days *in vitro* (DIV) neurospheres were plated onto ECM-coated glass, cultured a further 1-21 DIV, fixed and immunocharacterised. We also investigated the potential of injected O4<sup>+</sup> OPCs to integrate within an aged retina model. **Results** - Neurospheres differentiated into all three neural phenotypes: neuronal, astrocytic and oligodendrocytes. Human OPCs first expressed O4 followed by O1, which were then both downregulated as the more mature marker GalC was expressed. Neurospheres were positive for A2B5, GD3 and O4 but not O1 (expressed at or beyond 28 DIV). Additionally, we demonstrate that the BMP4 antagonist noggin blocked the autocrine loop present in developing neurospheres, increasing the numbers of OPCs in first generation neurospheres. And 21 days post-transplant, transplanted HuNu<sup>+</sup> cells had incorporated into the aged rat retina. **Conclusions** - Spinal cord-derived neurospheres generate OPCs that are further increased by noggin treatment, and we demonstrate the critical effect of time on neurospheres before *in situ* generation of the mature phenotype. We then showed that injected OPCs viably incorporate into the neural architecture of the aged CNS. Our methods have further scope for investigation in other CNS disease assays and models.

## POS-MON-136

### DIFFUSION MR ANISOTROPY PREDICTORS OF CEREBRAL CORTICAL FOLDING IN A FETAL SHEEP MODEL

Geng G.<sup>1</sup>, Johnston L.<sup>2,3</sup>, Yan E.<sup>4</sup>, Britto J.<sup>3</sup>, Walker D.<sup>5</sup> and Egan G.<sup>2,3</sup>

<sup>1</sup>POWMRI. <sup>2</sup>UniMelb. <sup>3</sup>HFI. <sup>4</sup>NTRI. <sup>5</sup>Monash University.

**Purpose:** Understanding the biomechanisms of cerebral cortical folding is of fundamental importance for evolutionary theory and to ascertain the basis of cortical folding disorders such as microgyria and lissencephaly. In this study, we have used fetal sheep brains as a model of cortical folding and performed diffusion MRI across key gestational time points. Results demonstrate correspondence between cortical growth, heterogeneous white matter and the development of sulci. **Method:** Twelve direction high resolution (0.258 × 0.258 × 1 mm<sup>3</sup>) diffusion MRI data (b-value = 1000 s/mm<sup>2</sup>, TE/TR = 50ms/4s) were acquired of a fetal sheep brain at each of 60, 70, 80, 90 days gestation (dg), across which period the development of primary cortical folds occurs. The mean fractional anisotropy (mFA) was manually delineated in the subcortical white matter along skeletons parallel to the boundary between cortex and white matter. Cortical growth was measured by calculating cortical volume and surface area. **Results:** mFA at 60dg was markedly lower at the points where the cingulate and sylvian sulci subsequently appeared at 70dg. Similarly, mFA at 70dg was markedly lower at the points where the inferior sulcus formed at 80dg. At 80dg, mFA was markedly lower at the points where the middle and lateral sulci appeared at 90dg. The cortical volume and surface area increased linearly over the 60-90dg. **Conclusion:** Novel use of diffusion MRI data suggests that low anisotropy white matter is a predictor of the location of primary sulci. The white matter heterogeneity may be the result of localised cortico-cortico and cortico-thalamic connectivity and varying degrees of myelination, causal factors to be examined further by histological analysis.

## POS-MON-137

**A COMPUTATIONAL MODEL OF THE PATTERNS OF GENE EXPRESSION UNDERLYING CORTICAL AREA DEVELOPMENT**Giacomantonio C.E.<sup>1</sup> and Goodhill G.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, The University of Queensland. <sup>2</sup>School of Mathematics and Physics, The University of Queensland.

The cerebral cortex is divided up into many functionally distinct areas. The emergence of these areas during cortical development is dependent on the expression patterns of several genes. Along the anterior-posterior axis, gradients of Fgf8, Emx2, Pax6, Coup-tf1 and Sp8 play a particularly strong role in specifying areal identity. However, our understanding of the regulatory interactions between these genes that lead to their confinement to particular spatial patterns is currently qualitative and incomplete. We therefore used a computational model of the interactions between these five genes to determine which interactions are necessary and sufficient to create the anterior-posterior expression gradients observed experimentally. The model treats expression levels as Boolean, reflecting the qualitative nature of the expression data currently available. We simulated gene expression patterns created by all possible networks containing the five genes. The networks that produce patterns best matching those seen experimentally have several common features, indicating which interactions are critical to correct gene expression patterning. For instance our results show that repressive interactions are critical, but mutual repression loops are not, and that some of the interactions between genes that have been previously hypothesised to exist in fact degrade the performance of the network. Overall our model illuminates the design principles of the gene network regulating cortical area development, and makes novel predictions which can be tested experimentally.

## POS-MON-139

**CORTICAL DEVELOPMENT IN THE Tc1 MOUSE; A MODEL OF DOWN SYNDROME**Haas M.<sup>1</sup>, Tybulewicz V.<sup>1</sup>, Fisher E.<sup>2</sup> and Guillemot F.<sup>1</sup><sup>1</sup>National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. <sup>2</sup>University College London, Queen Square, London WC1N 3BG.

Down Syndrome (DS) results from gene dosage imbalance caused by trisomy of human chromosome 21 (Hsa21). The spectrum of phenotypes includes decreased cognitive ability in all cases, which is thought to arise from defects in proliferation, migration and differentiation of neurons during cerebral cortex development. We have used a transchromosomal mouse line which carries a freely segregating copy of Hsa21, the Tc1 mouse, to study a range of aspects of cortical development in DS. We identified no significant difference between the overall size of the Tc1 telencephalon mid-development, compared with wildtype littermates (n=25). BrdU labelling of dividing cells did not suggest any impairment of cell proliferation in Tc1 embryos at any time from E11.5 to E17.5, and four days after BrdU application there were no differences in BrdU labelled cells in the Tc1 upper cortical layers, suggesting no defect in radial migration. However, an increase in the migrating calbindin positive interneuron population was observed, particularly prior to E14 (n=6). In Tc1 adults, we did not identify any cell proliferation defects in the subgranular zone of the hippocampal dentate gyrus in 3 month-old mice (n=11). In ongoing studies, we are using GFP *in utero* electroporation to study dendrite morphology in Layer II/III projection neurons. Initial investigations suggest that dendrite branching is reduced, but dendritic spine density is not altered in the motor cortex of Tc1 mice at adolescence (n=5). These data indicate that neurogenesis is not impaired in the embryonic or adult Tc1 mouse, but migration and differentiation may be accelerated in this DS model.

## POS-MON-138

**NDFIP1 EXPRESSION IN THE CEREBRAL CORTEX**Goh C.P., Putz U. and Tan S.S.  
Florey Neuroscience Institutes.

Ndfip1 (Nedd4 family-interacting protein 1) is an adaptor protein for Nedd4 family E3 ligases. It facilitates the interaction of Nedd4 family E3 ligases to their substrates. Our unpublished data has shown that upregulation of Ndfip1 in the cortex compromised brain development. To understand the role of Ndfip1 in brain development, we studied the expression pattern of Ndfip1 in the cortex during various developmental stages. Ndfip1 expression is seen from embryonic day 11 (E11) to adult. The expression is higher during postnatal stages and peak at postnatal day 7 (P7). During early embryonic stages, Ndfip1 is mainly seen in the ventricular zone and the marginal zone. At E15 to E17, Ndfip1 is expressed throughout the cortex with higher expression in the marginal zone, cortical plate and the subplate. During postnatal stages, Ndfip1 is present throughout the cortex. In term of cell-type expression, Ndfip1 is present in pyramidal neurons, some of the interneurons but not astrocytes. The expression of Ndfip1 during developmental stages suggests that this protein might play a role in brain development.

## POS-MON-140

**NDFIP1 REGULATES CORTICAL NEURON NUMBERS AND IS REQUIRED FOR DENDRITIC COMPLEXITY**Hammond V.E.<sup>1</sup>, Howitt J.<sup>1</sup>, Gunnarsen J.M.<sup>1</sup>, Thomson R.<sup>1</sup>, Hyakumura T.<sup>1</sup>, Dixon M.P.<sup>2</sup>, Thomas T.<sup>2</sup>, Voss A.K.<sup>2</sup> and Tan S.-S.<sup>1</sup>  
<sup>1</sup>Florey Neuroscience Institutes, Melbourne, Australia. <sup>2</sup>Walter and Eliza Hall Institute, Melbourne, Australia.

Ndfip1 (Nedd4 family interacting protein 1), an adaptor molecule for the E3 ubiquitin ligase Nedd4, is expressed throughout the mouse brain during development and in the adult. To investigate the function of Ndfip1 during corticogenesis we used brains from transgenic mice that over-express Ndfip1 and those deficient in Ndfip1. Mice over-expressing Ndfip1 under the control of the  $\beta$ -actin promoter do not survive postnatally and their brains display severe developmental abnormalities. At embryonic day (E) 18 the brains are markedly smaller and this decrease in size is already evident at E13. Caspase3 immunohistochemistry and TUNEL analysis revealed massive apoptosis beginning at E12 and continuing until E16. BrdU birthdating studies and immunohistochemistry using the layer specific markers CTIP2 (lower layers) and Cux1 (upper layers) indicated that, while the entire cortical wall was thinner, the correct layers had formed. Neural progenitor specific- or forebrain pyramidal neuron-specific inactivation of the *Ndfip1* gene resulted in approximately 20% less neurons in the cortex at postnatal day 30 (P30) and morphologically abnormal neurons, with condensed nuclei and swelling of the cytoplasm. Rapid Golgi analysis of cortical neurons revealed diminished spine density. In conclusion, excessive levels of Ndfip1 during cortical development result in neuronal loss due to massive apoptosis, while lack of Ndfip1 leads to neuronal loss and reduced spine density. These pathologies point to roles for Ndfip1 in regulating cortical neuronal numbers and synaptic connectivity.

## POS-MON-141

**BRAIN DEVELOPMENT AND BEHAVIOUR IN A MOUSE MODEL OF DEVELOPMENTAL VITAMIN D (DVD) DEFICIENCY**Harms L.R.<sup>1</sup>, Eyles D.W.<sup>1,2</sup>, McGrath J.J.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup><sup>1</sup>Queensland Brain Institute, The University of Queensland.<sup>2</sup>Queensland Centre for Mental Health Research.

**Purpose:** Epidemiological evidence suggests that low maternal vitamin D may be a potential risk factor for several neuropsychiatric disorders, including schizophrenia. The biological plausibility of this proposal has been examined and verified using a developmental vitamin D (DVD)-deficient rat model. As genetic manipulations are more feasible in mice, the aim of this study was to perform a comprehensive screen in DVD-deficient mice to establish whether it is a suitable model to examine the role of vitamin D on brain development and behaviour. **Methods:** Briefly, female mice were fed a vitamin D-deficient diet from 6 weeks prior to conception until birth, and then transferred to a diet containing vitamin D. Control mice were fed a vitamin D-containing diet throughout the experiment. Brain tissue from the neonates was tested for forebrain gene expression using microarray, and brain morphology using MRI. Adult offspring were subjected to a comprehensive behavioural screen investigating many basic behavioural and cognitive domains and brains scanned using MRI. **Results:** Neonate DVD-deficient mice had altered gene expression in pathways related to brain development (reelin and neuregulin,  $P < 0.05$ ,  $n=8$ ), without any associated neuroanatomical changes. Adult DVD-deficient male mice had a subtle increase in striatal volume with a corresponding decrease in lateral ventricular volume ( $P < 0.05$ ,  $n=10$ ). DVD-deficient mice had a subtle behavioural phenotype, which included changes in locomotion in the home cage ( $n=15$ ,  $P < 0.05$ ) and altered exploration ( $n=10$ ,  $P < 0.05$ ). **Conclusions:** These studies show that DVD-deficiency leads to altered brain development and behaviour in a mouse model. Although the adult behavioural phenotype was subtle, low levels of vitamin D during gestation impacted on gene expression at birth. Therefore, the DVD-deficient mouse may be a useful model to further explore the mechanism by which vitamin D impacts on brain development.

## POS-MON-143

**EPHA4 INHIBITS NEURAL PRECURSOR PROLIFERATION IN THE ADULT HIPPOCAMPUS**Newcombe E.A.<sup>1</sup>, Li L.<sup>1</sup>, Spanevello M.D.<sup>2</sup>, Boyd A.W.<sup>2</sup> and Bartlett P.F.<sup>1</sup><sup>1</sup>Queensland Brain Institute, The University of Queensland, Brisbane.<sup>2</sup>Queensland Institute of Medical Research, Brisbane.

The production of new neurons in the adult hippocampus is functionally important for learning and memory. The Eph receptors and their ephrin ligands have recently been implicated in the regulation of neurogenesis in both the developing and adult mouse, with EphA4 being specifically identified as having a regulatory role in the apoptosis of neural precursors in the subventricular zone, another neurogenic region of the adult brain. Interestingly, EphA4 expression has also been found to decrease in the hippocampus prior to the onset of Alzheimer's disease (AD) symptoms. In this study, we demonstrate that the effect of EphA4 on neural precursors in the mouse hippocampus varies depending on age. We show that EphA4 is expressed on neural precursors in the developing hippocampus, and that blocking EphA4 activity leads to a  $17.2 \pm 6.7\%$  decrease in neural precursor number as measured by the neurosphere assay ( $n=3$  experiments). In contrast, blocking EphA4 in the adult hippocampus increases neural precursor number by  $89.5 \pm 19.4\%$  ( $n=5$  experiments), despite the receptor not being expressed on these precursors ( $n=3$  experiments). The latter finding supports the idea that EphA4 acts as an inhibitor of precursor activity in the adult hippocampus, as does the fact that an increase of  $24.9 \pm 3.7\%$  in proliferation was observed in the EphA4 knockout hippocampus ( $n=3$  animals), compared to wild-type, age matched controls ( $n=4$  animals). Taken together, these results indicate that EphA4 appears to promote proliferation when expressed on neural precursors in the developing mouse, whereas it exerts an inhibitory effect in the adult, a finding that may have implications for our understanding of conditions such as AD.

## POS-MON-142

**OLFACTORY PROGENITORS RESPOND DIFFERENTIALLY TO GDNF AND NEURTURIN**

Kaplinovsky T. and Cunningham A.

Developmental Neurosciences Program, Faculty of Medicine, UNSW, Sydney.

GDNF and neurturin (NTN) support the development, differentiation and maintenance of many neuronal types. GDNF family ligands (GFLs) signal via a tetrameric receptor complex, with two GPI-anchored co-receptors (GFR $\alpha$ s) and two RET receptor tyrosine kinases. RET exists as two main isoforms, RET9 and RET51, potentially activating different biological pathways. GDNF binds preferentially to GFR $\alpha$ 1 and NTN to GFR $\alpha$ 2, although the reverse has been observed *in vitro*. We previously reported expression of GDNF, NTN, GFR $\alpha$ s and RET isoforms in olfactory neuroepithelium, a region of the nervous system characterised by ongoing neurogenesis. GFR $\alpha$ s showed differential expression, with mature olfactory neurones expressing GFR $\alpha$ 1, and progenitors and immature neurones expressing GFR $\alpha$ 2. In the current study we used an olfactory neurosphere culture system to define expression of the GFR $\alpha$ s and study the responses of progenitors to GDNF and NTN. Progenitors were harvested from neonatal rat turbinates ( $n=30$  per preparation) and olfactory neurospheres generated. Control spheres were compared to GDNF or NTN-treated spheres. After 7 days in culture, GDNF-treated spheres ( $n=40$ ) were larger than controls ( $n=40$ ,  $p < 0.0001$ ) and NTN-treated were larger than GDNF-treated ( $n=40$ ,  $p < 0.05$ ); unpaired two-tailed student t-test. GDNF promoted differentiation and migration of cells away from spheres whereas NTN caused more extensive proliferation. Immunocytochemistry showed most cells within treated spheres expressed RET9, while RET51 was expressed more peripherally, by cells with immature neuronal morphology. GFR $\alpha$ 2 expression paralleled RET9 and GFR $\alpha$ 1 was limited to more mature, peripheral cells. Based on these results, we propose GFR $\alpha$ 2/RET9 forms the functional signalling complex for GFLs in neurosphere progenitors, while RET51 functions in immature neurones. GDNF and NTN both stimulate olfactory progenitor proliferation and our results would support them mediating this effect via activation of the GFR $\alpha$ 2/RET9 complex.

## POS-MON-144

**IDENTIFICATION AND CHARACTERISATION OF NEW SIGNALLING PATHWAYS FOR RND PROTEINS DURING MOUSE BRAIN DEVELOPMENT**

Qu Z.D., Tan S.-S. and Heng J.I.

Florey Neuroscience Institutes, Melbourne, Australia.

Since their discovery, members of the Rnd family of atypical Rho-like GTP-binding proteins have been shown to be important for controlling cell proliferation and migration in fibroblasts, but their widespread expression within the developing central nervous system suggests an important role for these genes in the genesis and maturation of neurons of the brain as well. We recently discovered that Rnd2 is critical for controlling the initiation of migration and neurite outgrowth by newborn neurons of the embryonic cerebral cortex (Heng et al, Nature, 2008) but the underlying molecular mechanisms for these functions remain poorly characterised. To address this, we undertook a yeast 2-hybrid interaction screen for Rnd binding partners in order to identify signalling pathways that may be regulated by this protein. These studies have led to the cloning and characterisation of several novel downstream effector molecules that regulate cell migration and morphology through RhoA-dependent and RhoA-independent pathways.



## POS-MON-145

**SIRT1 SIGNALLING SUPPRESSES NEURONAL DIFFERENTIATION IN ADULT NEURAL PRECURSOR CELLS**

Saharan S., Jhaveri D. and Bartlett P.F.

The Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

It is now well established that in the neurogenic niches of the adult mammalian brain, new neurons and glial cells are continuously generated from a self-renewing and multipotent pool of neural precursor cells (NPCs). However, the mechanisms underlying these cell-fate choices still remain an intriguing enigma. Here, we examine the role of Sirt1, an evolutionarily conserved NAD<sup>+</sup>-dependent histone deacetylase, in modulating neuronal differentiation. Our results reveal that Sirt1 is expressed in the neurogenic niches of the adult brain, namely the subventricular zone and the hippocampus. Using the *in-vitro* neurosphere assay, we demonstrate that Resveratrol, a putative Sirt1 agonist, leads to a dramatic inhibition of neuronal differentiation with the percentage of differentiated neurospheres containing neurons decreasing to 19±2.5% as compared to 45±8.7% in control neurosphere cultures ( $p < 0.001$ ;  $n = 5$  experiments). Resveratrol mediates its inhibitory effect through a Sirt1-dependent mechanism as Sirt1 knockdown, through infection with recombinant lentivector expressing Sirt1-specific short hairpin RNA (Sirt1-shRNA), rescues the inhibition. Furthermore, abrogation of Sirt1 signalling in proliferating NPCs directs them specifically down a neuronal lineage as evidenced by a remarkable increase in the number of differentiated neurospheres containing neurons (78.9±9.3% Sirt1-shRNA neurospheres versus 45±3.8% control-shRNA neurospheres;  $p < 0.001$ ;  $n = 4$  experiments). Taken together these findings elucidate for the first time the role of Sirt1 signalling in regulating adult neurogenesis and reveal Sirt1 to be a key regulator of neural cell-fate choice.

## POS-MON-147

**THE MIGRATORY BEHAVIOR OF INDIVIDUAL NEURAL CREST-DERIVED CELLS IN THE EMBRYONIC GUT**Bergner A.J.<sup>1</sup>, Newgreen D.F.<sup>2</sup>, Young H.M.<sup>1</sup> and Enomoto H.<sup>3</sup><sup>1</sup>Department of Anatomy & Cell Biology, University of Melbourne.<sup>2</sup>Murdoch Childrens Research Institute, Royal Childrens Hospital, Parkville, Australia. <sup>3</sup>RIKEN Center for Developmental Biology, Kobe, Japan.

The neural crest-derived cells that colonize the developing gut probably migrate further than any cell population in the developing embryo. Previous time-lapse studies of migrating neural crest-derived cells in the embryonic mouse gut have revealed important information about the behavior of the cell population, but because the cells migrate in chains in close association with each other, the migratory behavior of individual cells could not be examined. In this study we performed time lapse imaging using gut explants from embryonic mice in which neural crest cells express the photoconvertible protein, Kikume. Although individual neural crest cells migrated with high cell-cell contact, individual cells did not retain the same neighbours for more than 2 hours. The directional persistence of individual migrating cells was lower than that reported for cranial neural crest cells. Although the population of crest-derived cells advances along the gut at around 35-40  $\mu\text{m}/\text{hour}$ , some individual cells migrating along pre-existing strands of the network migrated at ~100  $\mu\text{m}/\text{hour}$  over a 3 hour period. Some of these cells appeared to use axons as substrates. Individual cells behind the migratory wavefront were surprisingly active, but most migrated circumferentially. These studies show that the migratory behavior of neural crest-derived cells in the developing gut shows important differences from cranial neural crest cells and from neural crest cells *in vitro*.

## POS-MON-146

**ISOLATION OF HUMAN NEURON RESTRICTED PRECURSOR CELLS FROM FETAL SPINAL CORD DERIVED HISTOTYPIC SPHERES**Weible II M.W.<sup>1</sup> and Chan-Ling T.<sup>2</sup><sup>1</sup>Griffith University, Biomolecular and Physical Sciences, Nathan,QLD, 4111, Australia. <sup>2</sup>University of Sydney, Department of Anatomy and Histology, Sydney, NSW, 2006, Australia.

In this study we examined organogenesis of primary aggregate cultures from developing human spinal cord and show it is possible to generate histotypic spheres using human neuroepithelial stem (NS) cells. Data were collected from spinal cord specimens ( $n = 27$ ) aged 7.5-19.5 weeks gestation (WG). Samples were dissociated and expanded in neural basal media. Resultant spheres were reseeded at low cell density to form aggregates or reaggregated at high density, with or without leukaemia inhibitory factor (LIF). Generated spheres were either examined for cytoarchitecture; plated and their progeny examined; or dissociated and analyzed by fluorescent-activated cell sorting (FACS). The principal findings were that: (i) organotypic spheres can be formed from human NS cells which we named neural embryoid bodies (NEB) (ii) NS cells undergo sequential development in culture; (iii) passage via reaggregation decreases cellular senescence; (iv) tissue culture method (reaggregation), media condition (+LIF) and sphere size (200-500  $\mu\text{m}$  diameter) significantly affect histotypic sphere formation; (v) NEB cytoarchitecture is characterized by a surface layer of neuron restricted precursor cells (NRP); and (vi) BMPRII<sup>+</sup>NRPs were characterized as nestin<sup>+</sup>/vimentin<sup>-</sup>/GFAP<sup>-</sup>/NeuN<sup>+</sup>/MAP2a/b<sup>-</sup>/βIII-tubulin<sup>+</sup> and can be sorted by FACS. NEB cytoarchitecture appears to mirror aspects of the developing nervous system such as the sequential expression of lineage specific proteins, NRP migration and upregulation of BMPRII. Given that NS cells have properties that are highly dependent on species and region of isolation, our studies provide the first description of the isolation of human BMPRII<sup>+</sup>NRP cells which could have application in human spinal cord injury.

## POS-MON-148

**DIFFERENTIAL GENE EXPRESSION IN MIGRATING CORTICAL INTERNEURONS DURING MOUSE FOREBRAIN DEVELOPMENT**Faux C.H.<sup>1</sup>, Rakic S.<sup>2</sup>, Andrews W.<sup>2</sup> and Parnavelas J.G.<sup>2</sup><sup>1</sup>Centre for Neuroscience, The University of Melbourne, Australia.<sup>2</sup>Department of Cell and Developmental Biology, University College London, London, UK.

Gamma-aminobutyric acid (GABA)ergic interneurons play a vital role in modulating the activity of the cerebral cortex, and disruptions to their function have been linked to neurological disorders such as schizophrenia and epilepsy. These cells originate in the ganglionic eminences (GE) of the ventral telencephalon and undergo tangential migration to enter the cortex. Currently, little is known about the signaling mechanisms that regulate interneuron migration. We, therefore, performed a microarray analysis comparing the changes in gene expression between the GABAergic interneurons that are actively migrating into the cortex to those in the GE. We were able to isolate pure populations of GABAergic cells by fluorescent activated cell sorting of cortex and GE from embryonic brains of glutamate decarboxylase 67 (GAD67)-GFP transgenic mice. Our microarray analysis identified a number of novel genes that were upregulated in migrating cortical interneurons at both E13.5 and E15.5. Many of these genes have previously been shown to play a role in cell migration of both neuronal and non-neuronal cell types. In addition, several of the genes identified are involved in the regulation of migratory processes, such as neurite outgrowth, cell adhesion, and re-modelling of the actin cytoskeleton and microtubule network. Moreover, quantitative PCR and *in situ* hybridization analyses confirmed that the expression of some of these genes is restricted to cortical interneurons. This data, therefore, provides a framework for future studies aimed to elucidate the complexities of interneuron migration and, in turn, may reveal important genes that are related to the development of specific neurological disorders.

## POS-MON-149

**STAGE-SPECIFIC ROLES FOR MIR-134 IN NEURONAL PRECURSOR CELL SURVIVAL AND POST-MITOTIC NEURONAL MIGRATION**Gaughwin P.M.<sup>1</sup>, Yang H.<sup>2</sup>, Rigoutsos I.<sup>3</sup>, Lim B.<sup>4,5</sup> and Brundin P.<sup>1</sup><sup>1</sup>Neuronal Survival Unit, BMCA10, Lunds Universitet, 22184 Lund, Sweden. <sup>2</sup>Bioinformatics Institute, A\*STAR, Singapore. <sup>3</sup>Bioinformatics and Pattern Discovery Group, IBM Thomas J Watson Research.<sup>4</sup>Stem Cell and Developmental Biology, Genome Institute of Singapore, Singapore. <sup>5</sup>Harvard Medical School, Boston, MA, USA.

MicroRNA (miR) mmu-miR-134 is elevated in the embryonic mouse brain but its function during neural development remains unknown. We have used a combination of *in vitro* cell culture, *in utero* electroporation, and lentiviral gene delivery in the postnatal mouse brain to address the sequential roles of miR-134 in neural precursor cell (NPC) survival and post-mitotic neuronal migration. We demonstrate that, in undifferentiated neural precursors (N=4), miR-134 attenuates levels of a Bone Morphogenic Protein (BMP) antagonist and thereby modulates NPC survival and proliferation. miR-134 is up-regulated following neuronal migration from the embryonic ventricular zone (N=4-10 animals), and reduces growth-factor stimulated neuronal migration, in part, through modulation of a second, differentiated neuron-specific, transcript. These data indicate stage-specific roles for miR-134 in neural lineage progression during embryonic cortical development.

## POS-MON-150

**CHRONIC CONSTRICTION INJURY ALTERS THE IB4 BINDING CAPACITY OF SPARED NOCICEPTORS KNOWN TO EXPRESS IB4 BINDING SITES PRIOR TO INJURY**Gerke-Duncan M.B., Van Dantzig T., Rahman S., Skarratt N. and Walker S.  
School of Medical Sciences (Anatomy and Histology), University of Sydney, NSW, 2006, AUSTRALIA.

IB4 binds to a population of nociceptors. Immunohistochemical studies show that the number of IB4+ nociceptors decreases after nerve injury. It remains unclear whether this decrease is due to death of the IB4+ nociceptors or is a reflection of an injury-induced alteration in IB4-binding capacity. Given that IB4+ nociceptors play an important role in neuropathic pain sensory symptomatology it was of interest to clarify the underlying cause of the IB4+ nociceptor decrease in a neuropathic pain model. Rats were anaesthetised (n=10), both sciatic nerves exposed and injected with 2µl of 250µg/ml IB4. After 5 days survival right sciatic nerves were re-exposed and were either subjected to chronic constriction injury (CCI, n=5) or sham surgery (n=5). At 6 days post-injury rats were perfused, L4 ganglia removed and processed to visualise both internalised/traced IB4 and external IB4 binding-sites simultaneously on the same neurons. A decrease in the number of IB4 traced neurons was noted on the injured side of CCI rats compared to that of the uninjured side and compared to both sides of sham rats. Moreover, the reduced IB4 traced population after CCI exhibited a shift in the pattern of IB4 binding capacity with 30.4% showing strong binding, 31.3% medium and 38.3% weak binding capabilities compared to an average of 70% showing strong binding, 21% medium and 9% weak binding capacities on uninjured and sham sides. These results clarify that the overall decrease in IB4 binding reported post-injury is predominantly due to *alterations in IB4 binding capacity by spared IB4+ nociceptors* as well as neuronal loss.

## POS-MON-151

**DISTINCT MICRODOMAINS OF PUTATIVE GLUTAMATERGIC AND PEPTIDERGIC NOCICEPTORS IN LAMINA I OF MOUSE LUMBAR DORSAL HORN**Anderson R.L., Clarke J.N., Buckley N.C., Vilimas P.I., Haberberger R.V. and Gibbins I.L.  
Centre for Neuroscience, Flinders University, GPO Box2100 Adelaide SA 5001, Australia.

Glutamate is considered the primary neurotransmitter of small-diameter, presumptive nociceptive, neurons projecting to lamina I of the spinal dorsal horn. However most peptide-containing sensory neurons lack detectable expression of proteins considered essential for glutamate release. We sought to locate the endings of presumptive glutamatergic nociceptors in the superficial laminae of mouse lumbar spinal cord. To distinguish between intrinsic and primary afferent glutamatergic terminals in the spinal cord, *in vitro* anterograde tracing of lumbar dorsal roots was combined with immunohistochemistry for the vesicular glutamate transporter, VGLUT2. Neurobiotin (NB, 1%) was applied to six lumbar dorsal roots on one side of the spinal cord and the contralateral L3 dorsal root for 4 hours at 37°C (n=4 animals). Spinal cord sections were immunolabelled for VGLUT2 and calcitonin gene-related peptide (CGRP), and the distribution of NB-labelled terminals containing VGLUT2 and CGRP immunoreactivity was analysed by high resolution confocal microscopy, 3D quantification, Fourier transformations of spatial data, and cluster analysis. In the dorsal horn, NB-labelled terminals containing VGLUT2 but not CGRP were restricted largely to lamina I and were clustered into microdomains spatially distinct from microdomains enriched in CGRP terminals. VGLUT2 microdomains tended to be more superficial to CGRP microdomains. Only 17±6% of CGRP-immunoreactive NB-labelled terminals contained VGLUT2, whilst 40±7% of VGLUT2-immunoreactive NB-labelled terminals contained CGRP. The organisation of glutamatergic and peptidergic terminals into discrete clusters supports the hypothesis that dorsal horn neurons within lamina I may receive convergent synaptic inputs from separate populations of glutamatergic and peptidergic nociceptors.

## POS-MON-152

**CHANGES IN EXPRESSION OF SEROTONIN SYNTHESISING ENZYMES IN TRIGEMINAL GANGLIA OF CYCLING FEMALE MICE**Asghari R.<sup>1</sup> and Connor M.<sup>1,2</sup><sup>1</sup>Brain and Mind Institute, University of Sydney. <sup>2</sup>Australian School of Advanced Medicine, Macquarie University.

**Purpose** Serotonin (5-HT) and 5-HT receptors are important in the pathogenesis and treatment of migraine, a disorder with a markedly higher occurrence in females. Some forms of migraine are also strongly correlated with changes in sex hormone levels. The basis of this link is not firmly established, although it has been reported that tryptophan hydroxylase 1 (TPH1) levels in the trigeminal ganglion (TG) are regulated during the estrus cycle of mice (Berman et al, 2006). We examined changes in mRNA and protein levels involved in 5-HT synthesis across the estrus cycle. **Methods** 13-week-old female C57 BL/6 mice were used, estrus cycle stage was determined by vaginal smear. Mice were deeply anaesthetized, decapitated and the TG removed. mRNA levels of TPH1 and 2, aromatic amino acid decarboxylase (AADC) and the 5-HT transporter (SERT) were determined using RT-PCR, and normalized to the house keeping gene 3-phosphoglycerate. Protein samples were isolated from TG and TPH1 and AADC levels assessed by western blot using β-actin as a reference. **Results** RT-PCR showed an increase in TPH1, TPH2, AADC and SERT mRNA during proestrus compared to diestrus and estrus (n=8 each, P<0.05). Relative TPH1 protein levels were elevated in proestrus (7.41 ± 1.06, P<0.001 n=8) compared to diestrus and estrus (2.19 ± 0.25, 3.05 ± 0.2). AADC levels were higher in proestrus (4.78 ± 0.66, P<0.001 n=8) compared to diestrus and estrus (1.42 ± 0.15, 1.93 ± 0.18). **Conclusion** Our results show that enzymes involved in 5-HT synthesis are regulated across the estrus cycle in TG, but the role of oestrogen in this regulation remains unknown.

## POS-MON-153

### ABSENCE OF SPHINGOSINE KINASE 1 EXPRESSION CHANGES THE NEUROCHEMICAL CHARACTERISTICS OF CULTURED MURINE DORSAL ROOT GANGLION NEURONS BUT DID NOT CHANGE THE RESPONSE TO INFLAMMATION

Tam Tam S.<sup>1</sup>, Chegeni N.<sup>2</sup>, Gibbins I.L.<sup>1</sup>, Kress M.<sup>2</sup> and Haberberger R.V.<sup>1</sup>

<sup>1</sup>Centre for Neuroscience, Flinders University of South Australia.

<sup>2</sup>Division of Physiology, Medical University Innsbruck, Austria.

Sphingosine kinase 1 (Sphk1) generates the bioactive and pro-nociceptive lipid sphingosine 1-phosphate (S1P). S1P activates sensory neurons but it is not known if sources of the sphingolipid are extraneuronal or if S1P could act as an autocrine factor released from sensory neurons themselves. We used real-time quantitative RT-PCR, multiple labelling immunohistochemistry and in situ hybridisation (ISH) for the detection of Sphk1 in human and murine dorsal root ganglia (DRG) and primary cultured DRG neurons of wild-type and Sphk1-KO mice. Chronic inflammation was induced by subplantar injection of Complete Freund's Adjuvant (CFA). Sphk1 mRNA and protein were present in human (n = 2) and murine (n = 5) DRG. Sphk1 was lower expressed compared to the second isoform Sphk2 in murine DRG but ISH demonstrated its presence in nearly all neurons and satellite cells. Absence of Sphk1 expression (Sphk1-KO) or inhibition of Sphks (dimethylsphingosine) modulated the neurochemical profile (increase in CGRP/IB4+ neurons) in primary cultured DRG neurons (n = 3 cultures/condition) but had no influence on the mRNA expression levels of Sphk2 or the S1P receptors. Sphk1-deficient mice showed no difference compared with wild-type animals (C57/Bl6) in their heat response during chronic inflammation (n = 6). Our data suggest that the expression of Sphk1 is present in human and murine sensory neurons. S1P generated from Sphk1 in sensory neurons seems to determine the neurochemical phenotype in response to acute isolation but has no influence on the response of nociceptive sensory neurons to chronic inflammation.

## POS-MON-155

### THE EFFECTS OF DELAYED OECS TRANSPLANTATION ON PAIN RESPONSES AFTER DORSAL ROOT INJURY

Wu A., Lauschke J.L. and Waite P.M.E.

Neural Injury Research Unit, School of Medical Sciences, UNSW.

Deafferentation pain is frequently reported following brachial plexus avulsion, a condition that involves dorsal root injury (DRI). The ability of olfactory ensheathing cells (OECs) to modify DRI-mediated pain has never been explored. **Purpose:** This study aimed to test the efficacy of delayed OEC transplantation to alleviate tactile and thermal hypersensitivity that develops in the rat forepaw after 2-root DRI (Wu et al., 2009). **Methods:** Experiments were performed on 16 adult male AAW rats. All animals underwent C7 & C8 DRI. DRI was carried out under anesthesia with ketamine/xylazine mixture (100/10mg/kg IP). Dorsal roots were exposed unilaterally and crushed medial to the dorsal root ganglia. The rats were assigned to two groups: control animals received delayed injection of medium (dMED, n=6) and experimental animals had delayed OECs transplantation (dOECs, n=10) into the ipsilateral dorsal horn. Development of spontaneous pain behaviors, tactile allodynia and thermal hyperalgesia were assessed before and up to 9 weeks after DRI. Anatomical changes within the dorsal horn were examined immunohistochemically with markers for CGRP, IB4 and VGLUT1. **Results:** DRI-mediated allodynia/hyperalgesia within the affected forepaw was present from 2-9 post-injury in control animals and was alleviated by delayed OECs transplantation. At 9 weeks, reduction in the area of deep laminae was seen in control animals, accompanied by recovery of CGRP intensity and aberrant sprouting of VGLUT1-positive fibres into the superficial laminae. OECs transplantation reduced the area of both the superficial & deep dorsal horn laminae, but afferent fibre sprouting was the same as in control animals. **Conclusion:** Collateral sprouting of CGRP-positive afferents from adjacent segments and aberrant expansion of VGLUT1 afferents observed in control animals may play a role in the development of deafferentation pain following DRI. Delayed OEC transplantation ameliorates the development of pain, and seems to involve mechanisms other than afferent sprouting.

## POS-MON-154

### TRIGEMINAL AND SPINAL DORSAL HORN DISCONTINUITY AND AVIAN EVOLUTION

Krutzfeldt N.O.E. and Wild J.M.

University of Auckland, Department of Anatomy with Radiology, Private Bag 92019, Auckland, New Zealand.

It is generally considered that the sensory trigeminal system is a rostral continuation of the spinal sensory system, evidenced in part by the fact that the concentric spinal dorsal horn laminae of Rexed are replicated in the concentrically laminated trigeminal dorsal horn of the lower medulla. In the majority of avian species (e.g. *Gallus gallus*), however, the spinal dorsal horn laminae II and III are not concentric, but side by side, with II lying lateral to III (Woodbury, 1998). Curiously, however, this 'schizocerate' condition is not continued into the trigeminal dorsal horn, which maintains a concentrically laminated or 'leiocerate' organization (Puelles et al., 2007). We asked, therefore, where in the chicken spinal cord does the transition from a schizocerate to a leiocerate condition take place, and does the descending trigeminal tract make any contribution to the schizocerate condition of upper cervical segments? These questions were answered by immunohistochemistry and tract tracing of trigeminal and spinal nerves. From an evolutionary perspective, the distribution of both leiocerate and schizocerate morphotypes in most avian lineages suggests that morphology is an inadequate taxonomic marker. Furthermore, it cannot be determined on this basis which morphotype represents the ancestral or plesiomorphic character.

## POS-MON-156

### INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION IN THE PRIMARY OLFACTORY PATHWAY OF WILD TYPE, CX3CR1<sup>+/GFP</sup> AND CX3CR1<sup>GFP/GFP</sup> MICE FOLLOWING DAMAGE AND BACTERIAL CHALLENGE

Harris J.A.<sup>1</sup>, West A.K.<sup>1</sup>, Ruitenberg M.J.<sup>2</sup> and Chuah M.I.<sup>1</sup>

<sup>1</sup>Menzies Research Institute, University of Tasmania. <sup>2</sup>University of Queensland.

The olfactory pathway is a potential route for harmful organisms to reach the brain. Although rare, infections such as meningitis and encephalitis have been found to use this route. A central goal of our research is to investigate the immune barrier in the nose. The neuroprotective chemokine CX3CL1 (fractalkine) is involved in mediating cell adhesion and chemotaxis, and signals by binding to the G protein-coupled receptor CX3CR1 expressed on macrophages and microglia. CX3CL1 signalling can be neuroprotective by inhibiting excessive production of pro-inflammatory molecules. In this study, the nasal lining of wild type (C57/BL6), and transgenic mice in which one or both copies of the CX3CR1 gene have been replaced by an enhanced green fluorescent protein (GFP)-encoding gene, was unilaterally ablated by irrigation with 1% Triton-X solution. Fluorescently labelled bacteria were then administered into the nasal cavity and the expression of inducible nitric oxide synthase (iNOS) examined by immunofluorescence. No significant difference in the density of macrophages and iNOS-expressing cells was present in the olfactory mucosa of WT mice and mice lacking CX3CR1. Compared to the WT mice, CX3CR1<sup>+/GFP</sup> and CX3CR1<sup>GFP/GFP</sup> mice had a significantly lower density of macrophages in the glomerular and granular layers of the olfactory bulb. CX3CR1<sup>+/GFP</sup> mice had significantly more iNOS-expressing cells in the glomerular layer than CX3CR1<sup>GFP/GFP</sup> mice. Some of the iNOS-expressing cells in the glomerular layer of heterozygotes appear to be olfactory ensheathing cells. The findings suggest that the chemokine CX3CL1 and its receptor may play a role in regulating macrophage activation in the immunological defence of the olfactory pathway.



## POS-MON-157

### ADULT OLFACTORY PRECURSOR CELL PROLIFERATION AND DIFFERENTIATION IS MEDIATED BY THE NEUROPEPTIDE Y SIGNALLING PATHWAY

Doyle K.L., Hort Y., Shine J. and Herzog H.  
Neuroscience Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010 Australia.

The identification of factors that promote neurogenesis within the olfactory neuroepithelium can provide clues to the process of mammalian nervous system repair. Neuropeptide Y (NPY) is expressed in neurons and supporting cells of the olfactory system. NPY regulates neuroproliferation of olfactory, hippocampal and sub-ventricular zone precursor cells via the Y1 and Y2 receptors. Another member of this family of peptides is peptide YY (PYY) that is also expressed in neurons, though to a lesser extent. *In vivo* analysis of the olfactory neuroepithelium was performed to quantify the numbers of olfactory receptor neurons in wildtype (WT), Y1, NPY, PYY and NPYPYY knockout (Y1<sup>-/-</sup>, NPY<sup>-/-</sup>, PYY<sup>-/-</sup> and NPYPYY<sup>-/-</sup>) mice. Interestingly, the absence of NPY alone did not have the same effect on neuronal differentiation as the absence of both NPY and PYY. Further investigations of NPYPYY<sup>-/-</sup> and PYY<sup>-/-</sup> mice identified a significantly greater number of olfactory receptor neurons compared to WT, Y1<sup>-/-</sup> and NPY<sup>-/-</sup> mice ( $p < 0.0001$ ). Furthermore, NPY<sup>-/-</sup> mice had a significantly reduced number of mature olfactory receptor neurons ( $p < 0.05$ ). We have also examined the proliferation of olfactory neurospheres in primary olfactory precursor cell cultures isolated from WT, Y1<sup>-/-</sup>, NPY<sup>-/-</sup>, NPYPYY<sup>-/-</sup> and PYY<sup>-/-</sup> mice. The number of neurospheres that survive *in vitro* from NPY<sup>-/-</sup> are significantly reduced compared to WT controls at 3 weeks ( $p < 0.05$ ). Olfactory neurospheres from NPYPYY<sup>-/-</sup> and PYY<sup>-/-</sup> are significantly reduced compared to WT controls at 1, 2 and 3 weeks ( $p < 0.0001$ ). These results indicate an important role for the NPY signalling pathway in the proliferation and differentiation of adult olfactory precursor cells.

## POS-MON-158

### COMMUNICATION BETWEEN TWO NEUROGENIC ZONES IN THE ADULT MOUSE NERVOUS SYSTEM

Meedeniya A.C.B., Dwyer P., Chehrehasa F. and Mackay-Sim A.  
National Centre for Adult Stem Cell Research, Griffith University, Brisbane, Queensland, Australia.

There is ongoing neurogenesis in the subventricular zone of the adult brain which supplies interneurons to the olfactory bulb. There is also continuous neurogenesis in the olfactory epithelium supplying new olfactory sensory neurons whose axons terminate in the olfactory bulb. These axons synapse with tyrosine hydroxylase positive periglomerular neurons within the olfactory bulb, which are the product of subventricular zone neurogenesis. We hypothesise that focal denervation of the olfactory sensory neurons and thereby lesioning of the presynaptic input to the Type 1 neurons would result in their degeneration, and a subsequent upregulation of subventricular zone neurogenesis. Adult mice ( $n=26$ ) were treated with methimazole causing the ablation of the olfactory epithelium, and the tissues examined at multiple time-points after treatment. The survival of the olfactory sensory neurons within the olfactory epithelium was assessed together with their terminals within glomeruli of the olfactory bulb. The loss of tyrosine hydroxylase periglomerular neurons was quantified. Cell proliferation in the subventricular zone was also quantified using an antibody against Ki67, a marker of proliferating cells, and EdU, a thymidine analogue to track cell proliferation. Methimazole treatment led to loss of olfactory sensory neurons in the olfactory epithelium, loss of their terminals in the glomeruli and loss of tyrosine hydroxylase positive periglomerular neurons in the olfactory bulb 14-18 days later ( $p=0.05$ ). Cell proliferation in the subventricular zone was increased 14 days post methimazole treatment ( $p=0.02$ ). The results are consistent with our hypothesis that neurogenesis in the brain has a common neurogenic axis with the olfactory neuroepithelium. We propose the presence of a signalling pathway between these two neurogenic zones, which remains to be elucidated.

## POS-MON-159

### IMMUNOLocalISATION OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) AND RECEPTOR TRKB IN THE HUMAN BRAINSTEM MEDULLA

Tang S.<sup>1,3</sup>, Machaalani R.<sup>2,3</sup> and Waters K.A.<sup>1,3,4</sup>  
<sup>1</sup>Department of Paediatrics and Child Health, University of Sydney, NSW 2006, Australia. <sup>2</sup>Department of Medicine, Room 206, Blackburn Building D06, University of Sydney, NSW 2006, Australia. <sup>3</sup>Bosch Institute, The University of Sydney, NSW 2006, Australia. <sup>4</sup>The Children's Hospital, Westmead Sydney, NSW 2145, Australia.

Brain-derived neurotrophic factor (BDNF) and its receptor TrkB are essential in promoting normal development of the central nervous system, with key roles in respiratory control, coordination of movement and balance, and feeding activities. Expression of these markers have not been previously studied in the human infant. This study provides a detailed account of the distribution and localisation of pro- and recombinant human- (rh) forms of BDNF, and of TrkB in the human infant brainstem medulla, with qualitative comparison to the expression in the human adult. It is hypothesised that all markers will be present in the studied nuclei and that the expression of BDNF and TrkB will be higher during development compared to adulthood. Using commercially available antibodies, we applied immunohistochemistry on formalin fixed and paraffin embedded human brainstem tissue [ $n=8$  for infant,  $n=6$  for adult], and qualitatively analysed the expression of proBDNF, rhBDNF and TrkB. Amongst the medulla nuclei studied, the highest expression of the markers was in the inferior olivary nucleus and arcuate nucleus. Lowest expression was in the nucleus of the solitary tract. Comparison between infants and adults showed higher expression in the infant brainstem nuclei of the hypoglossal, vestibular, and cuneate for all the studied markers. We conclude that BDNF and TrkB play important roles in development and control of respiration, movement, balance and feeding. Expression of the TrkB receptor is age-sensitive showing highest expression during early development.

## POS-MON-160

### PERIPHERAL AND CENTRAL PROJECTIONS OF MID-SIZE SENSORY NEURONS CONTAINING CALCITONIN GENE-RELATED PEPTIDE BUT NOT SUBSTANCE P IN MICE

Kestell G., Anderson R.L., Clarke J.N., Haberberger R.V. and Gibbins I.L.  
Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

Many small diameter sensory neurons in dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). These neurons generally have a nociceptive function. However, in DRG of mice, a population of mid-diameter neurons express CGRP but not SP. The projections and functions of these neurons are not known. Therefore, we have used multiple-labelling immunohistochemistry and axonal tracing with Neurobiotin *in vitro* to map the projections of these neurons from the cervical spinal cord to the forelimb. Mice (C57/Bl6) were anaesthetised with a lethal dose of inhaled isoflurane, prior to removal of the upper spinal cord, brachial plexus, dorsal root ganglia and skin of the fore paws. For pathway tracing ( $n=3$ ), Neurobiotin was applied to the C7 ventral ramus and the brachial plexus-spinal cord was incubated for 4 hours *in vitro*. Neurobiotin was detected with streptavidin-Cy3 or -DTAF in spinal cord and DRG that were also labelled for CGRP and SP. Skin was labelled with antibodies to CGRP, SP and neuron specific enolase (NSE). In paw skin ( $n=3$ ), varicose fibres containing CGRP but not SP were most prominent within dermal papillae of glabrous skin and around hair shafts in hairy skin. Within cervical spinal cord ( $n=4$ ), fibres containing CGRP were prominent in the superficial dorsal horn (lamina I) and deeper dorsal horn (lamina IV). CGRP fibres lacking SP were most prominent in lateral areas of lamina I and in lamina IV. These data suggest that DRG neurons with CGRP but not SP have multiple somatotopic projections consistent with a polymodal mechanoreceptor function.

## POS-MON-161

**IDENTIFICATION OF GLYCINERGIC NEURONS IN GUINEA PIG COCHLEAR NUCLEUS AND THEIR CONNECTIONS WITH AUDITORY BRAINSTEM CIRCUITRY**

Walsh L., Robertson D. and Mulders W.  
The University of Western Australia.

The presence of large multipolar glycinergic neurons in the mammalian cochlear nucleus has been known for many years (Alibardi, 1998). These neurons, which are believed to correspond to the "onset-chopper" response type described in physiological recordings, project to a variety of targets in the ipsilateral and contralateral cochlear nuclei and higher centres in the brainstem and they are thought to play an important role in auditory signal processing. What is still contentious is to what extent these neurons receive descending inputs from auditory efferent pathways (Mulders et al, 2009). Our long term goal is to answer this question by combining anatomical tracing and physiological recordings from single neurons in the guinea pig cochlear nucleus. Here we report results combining immunolabeling for glycine with anterograde labeling of synaptic inputs to glycinergic neurons in guinea pig cochlear nucleus. Strong, selective labeling of large neurons in cochlear nucleus was achieved using an antibody directed against paraformaldehyde-fixed tissue (courtesy D Pow). Anterograde labeling in the same sections was achieved using labeled dextran amine injected into nuclei of origin of descending pathways. The results show successful labeling of synaptic inputs to specific glycinergic cell populations in the cochlear nucleus. Alibardi L. (1998) *Ann Anat.* 180:427-38. Mulders et al (2009) *Hear Res.* 256:85-92.

## POS-MON-163

**COCULTURES OF STEM CELL-DERIVED NEURAL CREST-LIKE PROGENITORS WITH COCHLEAR EXPLANTS**

Nayagam B.<sup>1</sup>, Edge A.<sup>2</sup> and Dottori M.<sup>1</sup>

<sup>1</sup>The University of Melbourne. <sup>2</sup>Harvard University, Boston.

Low numbers of auditory neurons (ANs) are believed to compromise the clinical performance of a cochlear implant (CI). The focus of our research is to determine whether stem cells can be used to replace the ANs lost following deafness. In order to successfully replace ANs, stem cells must be capable of directed differentiation toward a sensory neural lineage, of organised outgrowth of processes, and of forming functional connections. We have developed an in vitro assay to test these parameters using cocultures of cochlear explants and human embryonic stem cells (hESCs). Specifically, hESC-derived neurospheres were differentiated towards neural crest-like cells using noggin and Y-27, and then cocultured with cochlear explants isolated from early post-natal day three rats. The ENVY line of ESCs were used, which express high levels of green fluorescent protein (GFP), enabling discrimination from the explant tissue following analysis. In all cases (n=8), hESC-derived progenitors differentiated into neurons and extended their processes towards (never away from) the explant. The GFP positive processes were observed to grow along the endogenous peripheral processes of the explant toward the sensory hair cells. This data suggests that hESC-derived neurons may be able to extend along and follow established neuronal pathways. The described assay will now be used to quantify the number of synapses formed from hESC-derived neural crest cells in vitro and whether connectivity can be improved using different drugs. These results will inform our in vivo transplantation studies into the deaf mammalian cochlea, which are aimed at testing whether stem cell transplants can improve hearing thresholds with a CI.

## POS-MON-162

**DEVELOPMENTAL REGULATION OF TRPC3 EXPRESSION IN THE MOUSE COCHLEA**

Phan P.A.B.<sup>1</sup>, Tadros S.F.<sup>1</sup>, Kim Y.<sup>1</sup>, Birnbaumer L.<sup>2</sup> and Housley G.D.<sup>1</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences, UNSW, Sydney, Australia. <sup>2</sup>Laboratory of Neurobiology, NIEHS/NIH, Research Triangle Park, NC, USA.

Canonical transient receptor potential (TRPC) non-selective cation channels assemble from TRPC subunits and exhibit multiple activation mechanisms. TRPC3 has been proposed as a Ca<sup>2+</sup> entry channel responsible for Ca<sup>2+</sup> homeostasis in cochlear hair cells. The present study determined the spatiotemporal profile of TRPC3 expression during cochlear ontogeny in the mouse. TRPC3 immunofluorescence of cryosectioned cochleae was performed using E16-adult tissue. We found that prior to birth, TRPC3 expression was strongest in the epithelial cells that establish scala media, particularly the sensory hair cell region (E16-E20; n=7). From early post-natal period, to the onset of hearing (P1-P12; n = 11), immunofluorescence was strongest in the hair cells, with increased expression in the stria vascularis and Reissner's membrane. Neurite labeling in the inner spiral plexus and outer spiral bundles developed perinatally, and signal in the spiral ganglion neuron (SGN) somata increased. Compared with the late embryonic / early post-natal levels, hair cell expression was relatively weaker in the third post-natal week, whereas SGN somata labeling was stronger. In the adult, TRPC3 expression was primarily in the soma of the SGN, the hair cells, and the outer sulcus cell region. Analysis of cochleae from TRPC3 knockout mice revealed no significant morphological differences (n=3), and auditory brainstem responses were normal to hyper-acute. This suggests that TRPC3 expression is not obligatory for cochlear development or sound transduction. These data particularly prompt investigation of the contribution of these ion channels to auditory neuron excitability.

## POS-MON-164

**AUDITORY NEURON SURVIVAL FOLLOWING IMPLANTATION OF ENCAPSULATED BDNF-EXPRESSING SCHWANN CELLS INTO THE DEAF GUINEA PIG COCHLEA**

Pettingill L.N.<sup>1</sup>, Wise A.K.<sup>1,2</sup>, Geaney M.<sup>3</sup> and Shepherd R.K.<sup>1,2</sup>

<sup>1</sup>The Bionic Ear Institute, Melbourne. <sup>2</sup>Department of Otolaryngology, The University of Melbourne. <sup>3</sup>Living Cell Technologies Ltd., Auckland, New Zealand.

**Purpose:** Auditory neurons, the target cells of the cochlear implant, undergo progressive degeneration in deafness. Importantly, exogenous delivery of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) produces pronounced protective effects on auditory neurons in animal models of deafness. However, a clinically applicable long-term delivery technique is required. Cell- and gene-based therapies have become potential therapeutic options for neurotrophin treatment. This study investigated the survival-promoting effects of encapsulated BDNF-expressing Schwann cells on auditory neurons in the deaf guinea pig. **Methods:** Schwann cells from P3 rat sciatic nerve were transfected with an expression plasmid encoding BDNF using Lipofectamine 2000 (Invitrogen), and these BDNF-Schwann cells were then encapsulated in a biocompatible alginate matrix (LCT, Ltd.). Normal hearing guinea pigs were systemically deafened, and five days post-deafening the scala tympani of the left cochleae were implanted with either encapsulated BDNF-Schwann cells (n=11) or empty (control) capsules (n=12). Auditory neuron survival was quantified two or four weeks post-implantation. **Results:** Ototoxin-induced deafening resulted in a profound hearing loss in all animals and subsequent auditory neuron degeneration that was compounded over time. In comparison to the control (empty capsule-implanted) cochleae, there was a clear trend, although not statistically significant, for greater auditory neuron survival following implantation of the encapsulated BDNF-Schwann cells. Interestingly, although the capsules were implanted into the basal turn, no localised effects were observed, with cell rescue apparent throughout all cochlear turns. **Conclusion:** The trends observed in this study suggest that implantation of neurotrophin-producing cells, such as these BDNF-Schwann cells, into the deaf cochlea has the potential to reduce the degenerative changes that normally occur. Furthermore, enhanced auditory neuron survival would be expected when such techniques are combined with chronic electrical stimulation.

## POS-MON-165

**EXPRESSION PATTERNS OF SOMATOSTATIN RECEPTORS SUBTYPES SST1 AND SST2 SUGGEST IMPORTANT FUNCTIONAL ROLE IN AUDITORY HAIR CELLS**Radojevic V.<sup>1,2</sup>, Setz C.<sup>1,2</sup>, Brand Y.<sup>1,2</sup>, Hanusek C.<sup>1,2</sup> and Bodmer D.<sup>1,2</sup><sup>1</sup>Department of Biomedicine, University Hospital Basel, Petersgraben 4, CH-4031, Basel, Switzerland. <sup>2</sup>Klinik fuer Ohren-, Nasen-, und Halskrankheiten, University Hospital, Petersgraben 4, CH-4031, Basel, Switzerland.

Sensorineural hearing loss is one of the most common disabilities in our society today. In our previous work we have detected expression of the mRNA of somatostatin receptor sst1 and sst2 within the cochlea. Most importantly, we found improved hair cell survival in somatostatin treated samples that had been exposed to gentamicin demonstrating a protective effect of somatostatin. Here we studied the expression of somatostatin and its sst1 and sst2 receptors in the mouse cochlea. Sst1 immunoreactivity was detected after 3 days in cultures (n=5) of dissociated postnatal mouse (P5) organ of Corti cells. Staining with the hair cell marker myosin 7A together with staining for sst1 revealed a perinuclear localization of sst1 in hair cells. In paraffin sections of the cochlea from postnatal (p5) and adult wild type mouse sst1 and sst2 receptors were located in inner as well as outer hair cells but also in the spiral ganglion. A similar expression of the sst1 and sst2 receptors in inner and outer hair cells was found in cultivated p6 mouse organ of Corti explants. In contrast, somatostatin was found to be expressed only in non-nervous tissue of the cochlea by staining and Western blot analysis. In order to further characterize the localization of somatostatin receptors in auditory hair cells, we have done double immunostainings with the presynaptic marker synaptophysin. At higher magnification, colocalization of sst1 and sst2 receptors with synaptophysin on outer and inner hair cells could be observed. Confocal microscopy confirmed the close association of synaptophysin with somatostatin receptors. These findings propose that the somatostatin signaling system may have a role in the maintenance or function of synapses in the auditory system.

## POS-MON-167

**SELECTIVE CHANGES IN EXPRESSION OF A POTASSIUM CHANNEL IN AN INNER EAR PUMPING EPITHELIUM**Layton M.<sup>1</sup>, Housley G.D.<sup>2</sup>, Rodger J.<sup>1</sup> and Robertson D.<sup>1</sup><sup>1</sup>The University of Western Australia. <sup>2</sup>The University of New South Wales.

K<sup>+</sup> channels play a crucial role in the stria vascularis, an ion transporting epithelium responsible for the unique ionic composition and electric potential of inner ear endolymph (Marcus and Shen, 1994). In this study we used qRT-PCR to investigate mRNA expression for one subunit (KCNQ1) of the K<sup>+</sup> channel subtype (KCNQ1/KCNE1) in primary cultures of guinea pig stria vascularis. Guinea pig specific primers were developed and expression levels were measured relative to mRNA levels of a ribosomal (S16) housekeeping gene. We found a dramatic and consistent reduction in relative level of expression of the potassium channel gene with time in culture, suggesting a specific down-regulation associated with the culture conditions. Purinergic receptors are thought to be involved in regulation of stria function (Housley et al, 2002). We therefore tested the hypothesis that the reduction of K<sup>+</sup> channel mRNA was the result of release of ATP and activation of purinergic receptors, by including the ATP hydrolyzing enzyme APyrase in the culture medium. When APyrase was present, there a trend to less reduction of K<sup>+</sup> channel expression for short times in culture although this was not statistically significant. For longer culture times, there was large inter-specimen variability with some cultures showing less and some more reduction of relative K<sup>+</sup> channel expression compared to controls. The mechanism of the observed selective reduction in stria K<sup>+</sup> channel expression requires further investigation. Housley GD et al (2002) *Audiol Neurotol* 27:55-61. Marcus DC, Shen Z. (1994) *Am J Physiol Cell Physiol* 267: C857-C864.

## POS-MON-166

**P2X<sub>2</sub> AND VILIP1 CO-EXPRESSION IN THE MOUSE COCHLEA AND VESTIBULAR SYSTEM**

Tadros S.F. and Housley G.D.

Department of Physiology, School of Medical Sciences, UNSW, Sydney.

The association between the neuronal calcium sensor VILIP1 and the P2X<sub>2</sub> receptor (an ATP-gated ion channel subunit) has been identified in the CNS. VILIP1 enhances ATP-gated inward current responses and trafficking of the channels to the plasma membrane. We investigated the possible contribution of VILIP1 to the regulation of ATP-gated ion channels in the mouse cochlea and vestibular system using immunofluorescence. Cochleae from adult C57BL/6J mice and P2X<sub>2</sub> knockout (P2X<sub>2</sub><sup>-/-</sup>) mice were fixed in PFA and cryosectioned after decalcification. Floating sections were immunolabeled using anti-P2X<sub>2</sub> (guinea pig anti-rat polyclonal antiserum; Neuromics) and anti-VILIP1 (rabbit anti-mouse, Abgent) primary antibodies. Secondary antibodies; Alexa Fluor 488 goat anti-guinea pig IgG and Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen) were used. Controls included pre-adsorption of VILIP1 antibody with the target peptide, which blocked the signal. VILIP1 co-expression with P2X<sub>2</sub> was confirmed in the cochlear spiral ganglion and the vestibular Scarpa ganglion neurons. In addition, VILIP1 expression was also found in the cytoplasm of the epithelial cells of the organ of Corti, except the outer hair cells, pillar cells and Deiters cells. P2X<sub>2</sub> receptor expression in these cells and all other cochlear partition cells (except the marginal cells of the stria vascularis) was most prominent at the endolymphatic face. In the vestibular crista ampullaris, VILIP1 was expressed in the hair cell stereocilia, while P2X<sub>2</sub> was immunolocalized at the cuticular plates and also throughout the dark cells. In the utricle, VILIP1 immunolabeling was in the stereocilia, whereas P2X<sub>2</sub> signal was diffuse on the endolymphatic surface of the hair cells. These data suggest that VILIP1 primarily contributes to the regulation of ATP-gated currents that affect auditory and vestibular neurotransmission.

## POS-MON-168

**NEUROTROPHINS AND AUDITORY NERVE FUNCTION**Sly D.<sup>1</sup>, Minter R.<sup>1</sup>, Heffer L.<sup>1</sup>, Hampson A.<sup>1</sup>, Li J.<sup>1</sup>, Nelson N.<sup>1</sup>, Manning E.<sup>1</sup>, Winata L.<sup>1</sup>, Shepherd R.<sup>2</sup> and O'Leary S.<sup>1</sup><sup>1</sup>Department of Otolaryngology, The University of Melbourne. <sup>2</sup>The Bionic Ear Institute.

Neurotrophins can prevent the *structural* nerve damage that normally accompanies deafness, and may be soon be used as an adjunct or replacement treatment for profound or partially deaf cochlear implant patients. However, before these agents are used clinically we believe their effect on nerve *function* requires investigation. In this series of studies, we examined the effect of neurotrophin treatment in normal hearing and chemically deafened (for one week) adult guinea pigs (n=30). Animals were then implanted with a mini-osmotic pump connected to a cannula to deliver brain derived neurotrophic factor or vehicle to the cochlea for four weeks. After treatments, auditory function of deafened animals was assessed by electrophysiological recordings of auditory nerve fibers in response to electrical pulse-trains delivered at rates up to 200 pulses per second and auditory function of normal hearing animals was assessed by otoacoustic emissions and auditory brainstem responses. Neurotrophin administration had a normalising effect on most measures in deafened animals, including threshold and dynamic range, however the latency of auditory nerve fibre responses was greatly reduced. In normal hearing animals, neurotrophin administration reduced the hearing loss cause by cochlea implant surgery and did not cause any adverse effects. These findings indicate nerve growth factors appear to normalise or preserve many responses of auditory nerve fibers, while the latency of the responses appear to be abnormal.



## POS-MON-169

### POST-EXPOSURE ADMINISTRATION OF ADENOSINE RECEPTOR AGONISTS MITIGATES NOISE-INDUCED COCHLEAR INJURY

Wong A.C.Y.<sup>1</sup>, Guo C.X.<sup>1</sup>, Lee K.H.<sup>1</sup>, Gupta R.<sup>1</sup>, Housley G.D.<sup>1,3</sup>, Thorne P.R.<sup>1,2</sup> and Vlajkovic S.M.<sup>1</sup>

<sup>1</sup>Department of Physiology, <sup>2</sup>Discipline of Audiology, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand.

<sup>3</sup>Department of Physiology, University of New South Wales, Sydney, Australia.

In many tissues, endogenous adenosine concentrations increase in response to cellular injury, offering protection against tissue damage. Here, we report that the activation of adenosine receptor signalling can mitigate cochlear injury after exposure to noise. In this study, Wistar rats (8-10 weeks) were exposed to broadband noise (110 dB SPL for 24 hours) to induce permanent threshold shift. Adenosine and selective adenosine receptor agonists (CCPA, CGS-21680 and CI-IB-MECA) were applied to the round window membrane of the cochlea six hours post-exposure. Hearing function was assessed by auditory brainstem responses (ABR) before and 48 hours after exposure. A partial threshold recovery (up to 20dB) was observed in the cochleae treated with adenosine and the selective A<sub>1</sub> adenosine receptor agonist CCPA. No threshold recovery was observed with CGS-21680 or CI-IB-MECA, the selective A<sub>2A</sub> and A<sub>3</sub> adenosine receptor agonists respectively. Free radical damage generated in the noise-exposed cochlea, as demonstrated by nitrotyrosine immunoreactivity, was reduced by adenosine and CCPA administration. We further investigated the effect of adenosine amine congener (ADAC), a selective adenosine A<sub>1</sub> receptor agonist devoid of peripheral side effects, on noise-induced cochlear injury. ADAC was administered intraperitoneally (100 µg/kg/day) at time intervals after noise exposure (8-12 kHz, 110 dB SPL for 2 or 24 hours, n=8/group). Hearing thresholds were assessed by ABRs and hair cell loss evaluated by quantitative histology. ADAC administration led to substantial threshold recovery (25-30dB), supported by increased sensory hair cell survival and reduced nitrotyrosine immunoreactivity. Our studies pinpoint A<sub>1</sub> adenosine receptors as prospective pharmacological targets to mitigate noise-induced cochlear injury.

## POS-MON-171

### TRACKING THE EXPRESSION OF GAD-67 AND GABAA RECEPTOR $\alpha 1$ SUBUNIT IN MULTIPLE NUCLEI OF THE AUDITORY PATHWAY FOLLOWING NOISE-INDUCED HEARING LOSS

Browne C.J., Kueh S.L., Morley J.W. and Parsons C.H.  
The University of Western Sydney, NSW, Australia.

Manipulations that produce a cochlear hearing loss result in a range of changes in key auditory nuclei at different levels of the brain. Changes include plasticity of tonotopic representation, changes in the pattern of spontaneous activity and changes in the balance of excitatory and inhibitory transmitter systems. Moreover, damage to the cochlea frequently results in tinnitus, suggesting that some of these neuronal changes are involved in the generation of tinnitus. To determine which area(s) may be involved in the generation of tinnitus, we investigated the time-course of changes in the expression of Glutamic Acid Decarboxylase-67 (GAD-67) and the GABA<sub>A</sub> receptor  $\alpha 1$  subunit (GABA<sub>A</sub>R $\alpha 1$ ) in auditory cortex (AC), inferior colliculus (IC) and dorsal cochlear nucleus (DCN), in the month following exposure to a 16 kHz bandpass (1/10th octave noise (115 dB SPL). Male Long Evans rats (n=20) were unilaterally exposed to the damaging noise for 1-hour. At 0, 4, 8, 16 or 32 days the rats were euthanased, their brains were removed and processed for immunohistochemistry or western blot to identify GABA<sub>A</sub>R $\alpha 1$  subunit expression or GAD-67, which was subsequently quantified in the auditory cortex, inferior colliculus and cochlear nucleus. Over the course of the study period we saw significant increases in GABA<sub>A</sub>R $\alpha 1$  expression compared to controls, but the timing of these changes differed for each region. The increase was evident in the AC on day 8, then in the IC on day 16 and in the DCN on day 32. These changes may reflect an attempt to balance excitatory transmission, which is known to increase following noise-induced hearing loss.

## POS-MON-170

### THE P75 NEUROTROPHIN RECEPTOR PROTECTS PRIMARY AUDITORY NEURONS AGAINST ACOUSTIC TRAUMA IN MICE

Tan J.<sup>1</sup>, Clarke M.<sup>2</sup>, Barrett G.<sup>3</sup> and Millard R.<sup>2</sup>

<sup>1</sup>The Bionic Ear Institute, 384-388 Albert St, East Melbourne, VIC 3002. <sup>2</sup>University of Melbourne, Dept of Otolaryngology. <sup>3</sup>University of Melbourne, Dept of Physiology.

Survival of primary auditory neurons (PANs) is dependent on neurotrophic factors released by the organ of Corti. One of these neurotrophic factors is the mature brain-derived neurotrophic factor (BDNF) which binds to TrkB and the p75 neurotrophin receptor (p75NTR). In the adult inner ear, p75NTR is expressed weakly in PANs and cochlear Schwann cells whereas TrkB is robustly expressed in PANs. When the organ of Corti is damaged during trauma, p75NTR expression dramatically increases but TrkB expression declines in these cells. It is unclear what role p75NTR plays under these conditions although in other neurons, p75NTR induces their death when its extracellular domain binds to immature forms of BDNF. To elucidate this role, we challenged wild type mice (p75<sup>+/+</sup>) and mice lacking the neurotrophin-binding domain of p75NTR (p75<sup>-/-</sup>) with an acoustic tone of 130 dB SPL, 10 kHz for 2 hours. This produces a permanent auditory threshold shift > 40 dB SPL, damages the organ of Corti and causes secondary degeneration of PANs. After acoustic trauma, mice were maintained for 3, 6 and 9 weeks. Interestingly, survival of PANs in p75<sup>-/-</sup> mice was significantly compromised in all 3 timepoints when compared to wild type mice: 19% reduction after 3 weeks (n=6, Student's t-test, p=0.002), 33% reduction after 6 weeks (n=6, Student's t-test, p<0.001) and 29% reduction after 9 weeks (n=6-8, Student's t-test, p=0.002). Therefore, our data do not support a role of p75NTR as a death inducer in PANs but show its crucial role in protecting PANs. Its up-regulation is more likely a compensatory response to trap diminishing levels of neurotrophins.

## POS-MON-172

### IN VIVO DETECTION OF COCHLEAR INFLAMMATION USING MAGNETIC RESONANCE IMAGING

Le Floch J.<sup>1</sup>, Pontre B.<sup>3</sup>, Telang R.S.<sup>1,2</sup>, Tan W.<sup>1</sup>, Vlajkovic S.M.<sup>1</sup> and Thorne P.R.<sup>1,2</sup>

<sup>1</sup>Department of Physiology, University of Auckland, Auckland, New Zealand. <sup>2</sup>Section of Audiology, University of Auckland, Auckland, New Zealand. <sup>3</sup>Centre for Advanced Magnetic Resonance Imaging, University of Auckland, Auckland, New Zealand.

Inner ear inflammation is thought to contribute to the development of hearing loss and balance disorders. However, because of the location of inner ear tissues deep within the temporal bone it is difficult to determine the occurrence and pathological influence of dynamic inflammatory disease, without invasive techniques. Recent advances in magnetic resonance imaging (MRI) offer innovative opportunities for studying function and metabolism of the intact and damaged cochlea. Here, we report preliminary results of the in vivo monitoring of changes in vascular permeability in cochlear tissues associated with inflammation and noise-exposure using a 4.7Tesla MRI system and a contrast agent (Gadodiamide). Guinea pigs (n=4) and rats (n=6) were exposed to either broadband noise (110dB SPL for 4 hours) in a sound-attenuating booth or were sensitised by bacterial lipopolysaccharide (LPS, 0.8mg/kg) followed 24 hours later by bilateral intra-tympanic injection (LPS, 30µl/tympanum) to induce cochlear inflammation. Either immediately or up to 3 days after inducing inflammation anaesthetised animals were scanned. Using an MR sequence, T1-weighted images were acquired pre- (baseline images) and post Gd injection (0.3-1.5mmol/kg). Several sets of images were obtained to measure the rate of Gd uptake into cochlear tissues as an index of vascular permeability. Quantitative changes in signal intensity over time in the cochlea and other tissues were calculated. With LPS and noise in guinea-pigs, signal enhancement was found in cochlear tissues. Histology showed evidence of tissue inflammation. These data suggest that Gd uptake increased with cochlear inflammation and occurred as a consequence of increased vascular permeability. It demonstrates that MRI can monitor longitudinally the normal and inflamed cochlea in vivo.

## POS-MON-173

**PLZF-DEFICIENT MOUSE MUTANTS DO NOT GENERATE CONDITIONING-MEDIATED PROTECTION FROM ACOUSTIC TRAUMA**Peppi M.<sup>1,2</sup>, Kujawa S.G.<sup>1,2</sup> and Sewell W.F.<sup>1,2</sup><sup>1</sup>Otology and laryngology, Harvard Medical School, Boston MA.<sup>2</sup>Eaton-peabody Laboratory, Massachusetts Eye and Ear Infirmary Boston MA.

The cochlea can be "conditioned" to resist acoustic trauma via a corticosteroid-dependent process (Tahera et al, 2006). The amount of conditioning-related protection is remarkable; up to 40 dB of acoustic threshold shift can be prevented. The spectrum of damage in acoustic trauma ranges from excitotoxicity in afferent dendrites to apoptotic loss of hair cells, all of which can be prevented by conditioning. While many potential targets of corticosteroid activation have been analyzed in the ear, no compelling mechanism has yet been identified for its action. We have identified a transcriptional protein, PLZF, which is present in the spiral ganglion, organ of Corti, and spiral ligament, all targets for acoustic trauma. PLZF mRNA is elevated in the mouse cochlea following acoustic stimulation, restraint stress, and corticosteroid treatment. PLZF appears to play an essential role in conditioning resistance to acoustic trauma: mice deficient in PLZF have normal hearing and normal responses to acoustic trauma, but are unable to generate restraint-stress (conditioning) mediated protection against acoustic trauma.

## POS-MON-174

**INDIVIDUAL MICRO-RNA EXPRESSION IS DOWN REGULATED IN PRIMARY CULTURED SENSORY NEURONS AND CHANGES IN RESPONSE TO SUBSTRATE AND NGF**Bastian I.<sup>1</sup>, Tam Tam S.<sup>1</sup>, Gibbins I.L.<sup>1</sup>, Zhou X.F.<sup>1</sup>, Michael M.Z.<sup>2</sup>, Rogers M.L.<sup>1</sup> and Haberberger R.V.<sup>1</sup><sup>1</sup>Centre for Neuroscience, Flinders University of South Australia.<sup>2</sup>Gastroenterology and Hepatology, Flinders Medical Centre.

MicroRNAs (miRNAs) are small RNAs that control gene expression. More than 700 human miRNAs have been identified and only some of their functions in various physiological and pathological processes are known. In the nervous system miRNAs are implicated in regulating processes like neuronal differentiation, synaptic plasticity and neurodegeneration. We investigated miRNA-expression profiles in cultured sensory neurons, under different growth conditions. In particular we investigated the influence of extracellular matrix (ECM) components and nerve growth factor (NGF) on miRNAs in sensory neurons. Sensory neurons were obtained from dorsal root ganglia (DRGs) of 6 weeks old, male C57Bl6 mice (n=5). Based on the results of miRNA-microarray analysis, we found 7 miRNAs (miR-1, miR-34b, miR-142-3p, miR-143, miR-199a, miR-199a3p, miR-442b), which were significantly down-regulated after one day in culture. We quantified the relative and absolute miRNA-expression levels (d1-d5) using real-time PCR and localised miRNAs 1 and 199 via In-Situ-Hybridisation. Cells were grown on Poly-D-lysine and Poly-D-lysine/Laminin and the miRNA expression was analysed in presence and in absence of the NGF (n=3-5). Real-time PCR verified the down regulation of miRNAs in culture. The miRNA expression was modulated by substrate and presence of exogenous and endogenous NGF. Presence of laminin, which led to improved density and the outgrowth of processes, increased the miRNA expression levels. At d2, except miR-442b, all miRNAs were reduced in presence of NGF. In summary, our findings show that growth conditions (i.e. ECM and NGF) regulate miRNA-expression in sensory neurons and suggest that miRNAs are part of the response of sensory neurons to nerve damage and regrowth.

## POS-MON-175

**A NOVEL NEUROTROPHIC FACTOR SUPPORTS SPIRAL GANGLION NEURON SURVIVAL AND THEIR ELECTRICAL RESPONSIVENESS IN VIVO**Fransson A.E.<sup>1</sup>, Joergensen J.R.<sup>2</sup>, Kalkkinen N.<sup>3</sup>, Wahlberg L.<sup>2</sup> and Ulfendahl M.<sup>1</sup><sup>1</sup>Karolinska Institutet, Center for Hearing and CommunicationResearch, Stockholm, Sweden. <sup>2</sup>NsGene A/S, Ballerup, Denmark.<sup>3</sup>Institute of Biotechnology, University of Helsinki, Finland.

Meteorin-like is a virtually undescribed potential neurotrophic factor expressed in the inner ear during development (Ramialison et al., Genome Biol. 2008 Oct 1;9 (10):R145). To investigate the neurotrophic properties of Meteorin-like in relation to deafness, recombinant protein was produced and administered to deafened guinea pigs. Briefly, mouse Meteorin-like was cloned, expressed in mammalian cells and secretion verified by western blotting. Recombinant protein was subsequently purified and characterized by mass spectrometry. Next, sixteen animals were deafened by intracochlear infusion using 10% neomycin for 48 hours. They were divided into two groups, one group received Meteorin-like (1µg/ml) and the other group received artificial perilymph using a mini-osmotic pump. After two weeks treatment the pump was removed and the animal stayed in the study for another two weeks. Electrically-evoked auditory brainstem (eABR) was measured day 2, 7, 14, 21 and 28 counted from the time of the cochlear implant. After four weeks the eABR results showed a significant difference in favor for the Meteorin-like treated group. Cochleae are being processed for morphology analysis.

## POS-MON-176

**PERIPHERIN INHIBITS NEURITOGENESIS FROM TYPE II SPIRAL GANGLION NEURONS IN THE NEONATAL MOUSE COCHLEA IN VITRO**Barclay M.<sup>1</sup>, Julien J.P.<sup>2</sup>, Ryan A.F.<sup>3</sup> and Housley G.D.<sup>1,4</sup><sup>1</sup>Department of Physiology, The University of Auckland, Auckland,New Zealand. <sup>2</sup>Department of Anatomy and Physiology, LavalUniversity, Quebec, Canada. <sup>3</sup>Departments of Surgery &

Neuroscience, University of California San Diego and VA Medical

Center, La Jolla, CA, USA. <sup>4</sup>Department of Physiology & Translational

Neuroscience Facility, University of New South Wales, Sydney, Australia.

Peripherin is one of five intermediate filament proteins expressed in neurons and conclusive evidence for the function of this protein remains elusive. The mouse cochlea provides a means of investigating peripherin function due to its exclusive expression in a sub-population of primary auditory neurons, the Type II spiral ganglion neurons (SGNII), which innervate the outer hair cells (OHC). We investigated the effect of peripherin gene deletion on the development of the peripheral neurites of SGNII in vivo and in vitro at P1, a time when SGNII are undergoing extension.  $\beta$ -tubulin immunofluorescence distinguished all SGN and peripherin immunolabelling discriminated neurites arising from SGNII in WT tissue. Peripherin gene deletion did not discernibly affect the morphology of SGNII innervation of the OHC. However, 48 hour culture (in minimal growth media) of SGN explants from KO mice yielded a significant increase in the number of neurites/explant compared with explants from WT animals. This effect was obscured by the addition of 100ng/ml BDNF to culture medium, which greatly increased neurite number in WT and KO explants. The mean length of neurites in explants from KO mice was significantly longer than those from WT mice regardless of the presence of BDNF in the culture media, as was the length to turning. These results indicate that peripherin expression by SGNII inhibits neurite extension. This may affect the length of SGNII, and thus the location and/or number of OHC innervated by each nerve fibre in vivo.

## POS-MON-177

**PROTECTION OF SPIRAL GANGLION NEURONS WITH NEUROTROPHINS AND CHRONIC ELECTRICAL STIMULATION**

Wise A.K.<sup>1</sup>, Fallon J.B.<sup>1</sup>, Evans A.J.<sup>1</sup>, Andrew J.<sup>1</sup>, Pettingill L.N.<sup>1</sup>, Geaney M.S.<sup>2</sup> and Shepherd R.K.<sup>1</sup>

<sup>1</sup>The Bionic Ear Institute, 384-388 Albert Street, East Melbourne. <sup>2</sup>Living Cell Technologies, Auckland, New Zealand.

In the deaf cochlea spiral ganglion neurons (SGNs) undergo continual degeneration that ultimately leads to neuron death. The exogenous application of neurotrophins (NTs) can prevent SGN degeneration and even promote regrowth. Furthermore, combining chronic intracochlear electrical stimulation (ICES) with NTs can enhance the survival effects of NTs and lower electrical thresholds. However, following the cessation of NT delivery SGNs continue to degenerate. Therefore techniques that deliver NTs over a long period of time are required to maintain the therapeutic benefit of NT treatment. We have used cell-based therapy to provide NTs in combination with an intracochlear electrode array in a long-term deafened cat model. Cats were neonatally deafened with neomycin, and at two months of age were implanted with encapsulated porcine choroid plexus cells (NTCell, LCT Inc.) and the stimulating electrode array. The choroid plexus cells produce NTs and were encased in alginate capsules that enabled the diffusion of NTs into the cochlear fluids. Environmentally derived ICES was delivered chronically via a clinical stimulator (Nucleus CI24M, Cochlear™) and processor (Esprit 3G, Cochlear™). Five cats received chronic ICES only. Six cats received NTs without chronic ICES and six cats received NTs in combination with chronic ICES. Control animals (n=7) were normal hearing and were not implanted. The results indicated that chronic ICES alone (without NTs) did not provide greater SGN survival compared to the contralateral untreated cochlea. Importantly, chronic ICES in combination with NTs provided greater SGN protection than NTs alone or chronic ICES alone (ANOVA P<0.003). Treatment with NTs alone led to an improvement in thresholds from electrically evoked brainstem responses (ANOVA P<0.003). These results indicate that cell-based NT delivery in combination with ICES can promote SGN survival. These findings have important implications for future strategies that will combine cochlear implantation with systems that deliver drugs safely to the cochlea. This research was funded by The Garnett Passe and Rodney Williams Memorial Foundation and the US National Institutes of Health (HHS-N-263-2007-00053-C).

## POS-MON-179

**FREQUENCY DISCRIMINATION USING MICROSTIMULATION OF THE COCHLEAR NUCLEUS: A BEHAVIOURAL INVESTIGATION**

Morgan S.J. and Paolini A.G.

Graeme Clark Centre, La Trobe University, Bundoora, Australia.

This study investigated whether a fear could be conditioned in response to discrimination of acoustic tones of alternating frequency. Subsequently it was tested whether the response could be evoked by microstimulation of the cochlear nucleus. To replicate the effect of alternating tone frequency, different frequency-specific sites in the cochlear nucleus were electrically stimulated in a similar alternating format. Change in ECG in response to stimulus presentation verified the effectiveness of this paradigm for the detection of frequency discrimination, and was used as a measure of discriminability of both acoustic and electrical stimuli. Preliminary findings suggest that electrical stimulation did not achieve similar levels of discriminability as acoustic stimulation. This was irrespective of region of the cochlear nucleus stimulated, with stimulation locations confirmed using 3D modelling of X-Ray CT images, multiunit cluster response, and histology. This may reflect the complex mechanisms of the cochlear nucleus, and suggest that further investigation into stimulation strategies is warranted.

## POS-MON-178

**EFFECTS OF LONG-TERM DEAFNESS AND DELAYED CHRONIC INTRACOCHLEAR ELECTRICAL STIMULATION ON THE PRIMARY AUDITORY CORTEX**

Fallon J.B., Irvine D.R.F., Evans A.J., Landry T.G. and Shepherd R.K.  
The Bionic Ear Institute, Victoria, Australia.

Cochlear implant use from a young age is known to alter spectral (spatial) and temporal processing in the auditory system. Whether these effects are limited to electrical stimulation (ES) that is initiated during the early critical periods, or also occurs when ES is commenced after long-term deafness, is less clear. Five cats were neonatally deafened via daily neomycin injections, and at two months of age implanted a multi-channel scala tympani electrode array. Behaviorally relevant ES from a cochlear implant was delivered from *eight* to *fourteen* months of age. Neuronal clusters (n = 300) were recorded in the primary auditory cortex (AI) using a combination of single tungsten and multi-channel silicon electrode arrays. Spectral processing in AI was assessed by measuring the cochlea-to-cortex mapping and temporal resolution was quantified as the jitter in response latency and the maximum rate at which clusters could be driven. Similar to chronic ES initiated early in life, delayed ES had little effect on the basic response properties of AI neurons, but did reverse the disruption of the cochlea-to-cortex mapping and reduction in maximum driven rate (Mann-Whitney; p < 0.05) resulting from long-term deafness in the absence of CI use. The late initiation of ES did not, however, reverse the increase in the jitter in response latency seen with long-term deafness. We hypothesize that the inability of electrical activation of the cochlea, after the closure of the normal critical period, to reverse the increased jitter in response latency contributes to the poorer performance observed among congenitally deaf human patients implanted later in life.

## POS-MON-180

**TEMPORAL PROPERTIES OF DENDRITIC PROCESSING IN OCTOPUS CELLS OF THE POSTEROVENTRAL COCHLEAR NUCLEUS**

Spencer M.J.<sup>1,2</sup>, Bruce I.C.<sup>1,3,4</sup>, Grayden D.B.<sup>1,4</sup>, Meffin H.<sup>1,2</sup> and Burkitt A.N.<sup>1,4</sup>

<sup>1</sup>Department of Electrical and Electronic Engineering, University of Melbourne, Victoria, Australia. <sup>2</sup>National ICT Australia, Victoria, Australia. <sup>3</sup>Department of Electrical and Computer Engineering, McMaster University, Ontario, Canada. <sup>4</sup>Bionic Ear Institute, Victoria, Australia.

Octopus cells in the posteroventral cochlear nucleus detect broadband auditory events by performing a coincidence detection across many (>60) Auditory Nerve Fibres (ANFs), covering ~1/3 of the ANFs' tonotopicity. It has been suggested that octopus cell dendrites introduce a delay to compensate for systematic variation in ANF spike latency, which is a known function of Characteristic Frequency (CF). In this study, numerical modelling (in NEURON and MATLAB) was used to calculate the Post-Synaptic Potential (PSP) propagation delay in octopus cell dendrites. The model's parameters were based on published experimental results from a number of papers dealing with cats, although the results are relevant for most mammals including humans. This study showed that an octopus cell dendrite with typical morphology could provide a PSP delay of  $0.5 \pm 0.2$  ms. The resulting compensation would allow coincidence detection of  $0.2 \pm 0.1$  octaves of the lowest CF ANFs, or  $3 \pm 0.5$  octaves of the highest CF ANFs. The uncertainty intervals are dominated by the imprecise knowledge of membrane properties of the dendrites of octopus cells and of the exact functional relationship between CF and spike latency in ANFs. These results support the hypothesis that the dendrites are providing a compensatory delay, however, the delay is not enough to allow for coincidence detection across 1/3 of the tonotopicity at low CF ANFs.



## POS-MON-181

**A DETAILED COCHLEAR NUCLEUS NETWORK MODEL: CONSTRAINING PARAMETERS USING EXPERIMENTAL DATA****Eager M.A.**<sup>1,2</sup>, Grayden D.B.<sup>3,2</sup>, Meffin H.<sup>4</sup> and Burkitt A.N.<sup>3,2</sup><sup>1</sup>Department of Otolaryngology, University of Melbourne. <sup>2</sup>The Bionic Ear Institute. <sup>3</sup>Department of Electrical and Electronic Engineering, University of Melbourne. <sup>4</sup>National ICT Australia.

**Introduction** Understanding the function of networks within the auditory pathway is essential for future developments in cochlear and brainstem implants. This study evaluated sequential optimisation techniques for determining the synaptic parameters of a biophysically-based neural network of the cochlear nucleus. **Methods** The detailed model was simulated in NEURON and the input was provided by the most recent auditory periphery model for high and low spontaneous rate ANFs. Sequential optimisation included the automatic fitting of individual parameters using experimental data. **Results** Experiment 1 constrained parameters for GABAergic golgi cells so that the rate-level output was less than 2% error of experimental data (error normalised to max. rate). Experiment 2 optimised the parameters controlling the adaptation to GABAergic input to D-stellate cells using experimental click recovery data (normalised rate for 2,4,8,16 ms click pairs) with final error 0.5 ms. Experiment 3 optimised synaptic parameters for Type II DCN units or Tuberculoventral cells, which receive wide-band inhibition from DS cells. The cost function used notch-noise stimuli and measures across a population of cells to match data from Reiss and Young (J Neurophys, 25, 3680-91, 2005). Experiment 4 used the experimental intracellular data in TS cells by Paolini and colleagues to find the parameter bounds for three classification types of chopper units: sustained, transient and transient-adapting. Important factors were HSR/LSR ratio and degree of inhibition from 3 cell types. **Conclusion** The advancements in computational neuroscience are enabling greater understanding of neural pathways and their underlying microcircuits. The development of the cochlear nucleus model furthers our understanding of the auditory pathway.

## POS-MON-182

**MIDBRAIN RESPONSES TO MICROSTIMULATION OF THE COCHLEA USING MULTI-CHANNEL THIN-FILM ELECTRODES****Allitt B.**<sup>1</sup>, Morgan S.J.<sup>1</sup>, Bell S.<sup>1</sup>, Nayagam D.<sup>2</sup>, Arhatari B.<sup>1</sup>, Clark G.M.<sup>1</sup> and Paolini A.G.<sup>1</sup><sup>1</sup>Graeme Clark Centre, La Trobe University, Bundoora, Australia. <sup>2</sup>Bionic Ear Institute, East Melbourne, Australia.

Thin-film microelectrodes were used to stimulate the cochlea of five urethane-anesthetised rats. Simultaneous recordings were taken from the central nucleus of the inferior colliculus over 160 possible multiunit clusters. Distance between stimulation and reference sites, stimulation current and phase duration was altered on the cochlear implant, and resulting changes in rate level functions, thresholds, and extent of neural activation in the IC were examined. Increases in distance between stimulation and reference site led to increased broadness of resulting activity in the IC. Similarity of electrically-evoked CIC activity to acoustic stimulation was substantially dependent on placement of the stimulating electrode within the cochlea. Furthermore, close proximity of the electrode to the modiolus influenced threshold for activity in the CIC. These results suggest higher density of potential stimulation sites on electrodes which permits finer control over charge delivery may permit improved frequency specificity in future cochlear implant devices.

## POS-MON-183

**IN VITRO EPIRETINAL STIMULATION USING A HEXAGONAL ELECTRODE ARRANGEMENT****Abramian M.**, Dokos S. and Lovell N.H.

Graduate School of Biomedical Engineering, University of New South Wales, Sydney, Australia.

The aim of this study is to characterise in vitro epiretinal stimulation, using a hexagonal electrode configuration, and to incorporate the experimental results into mathematical model of retinal activation. Electrical stimulation was performed on NZW rabbit retinas (n=7) using 7 platinum disc electrodes (0.125mm diameter) arranged in a hexagonal configuration. Ganglion cell action potentials were recorded using tungsten microelectrodes, with three types of responses obtained: 1) Single constant-latency spikes appearing immediately after the stimulus pulse. These responses were attributed to direct activation of ganglion cell axons. 2) Single spikes showing variable, long latencies. These spikes were most likely synaptic ganglion cell activation. 3) Single spikes with near-constant short latencies, with thresholds lower than axonal responses. These spikes are likely to be originated at the axon hillock because: a) they had lower activation thresholds, owing to known high sodium channel density in this region, and b) they were followed by long-latency responses, indicating proximity of the activated region to the ganglion cell receptive field. Thresholds were measured as a function of pulse duration (50-500µs) and distance, across (up to 0.15mm from electrode centre) and above (up to 0.1mm) the retina. Axonal activation thresholds were 12.4±3.2µA (n=4) for 0.1ms and 7.4±3.7µA for 0.3ms pulse durations (n=7). In two cells, type 2 and 3 spikes were recorded. Type 2 response thresholds were 4.8 and 4.3µA, and type 3 response thresholds were 8.1 and 8.4µA, with 0.1ms pulses. Strength-duration curves suggested that shorter pulses are more suitable for selective activation of the axon hillock. All thresholds increased markedly with distance from the activation site.

## POS-MON-184

**FOCAL ACTIVATION OF VISUAL CORTEX THROUGH SUPRACHOROIDAL ELECTRICAL STIMULATION OF THE RETINA****Hadjinicolaou A.E.**<sup>1</sup>, Hietanen M.A.<sup>1</sup>, Suaning G.J.<sup>2</sup>, Ibbotson M.R.<sup>1</sup> and Cloherty S.L.<sup>1</sup><sup>1</sup>ARC Centre of Excellence in Vision Science, Research School of Biology, Australian National University, Canberra, ACT. <sup>2</sup>Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW.

A retinal vision prosthesis is tasked with restoring vision by way of electrical stimulation of the retina left intact by degenerative diseases. Here we investigate activation of the primary visual cortex resulting from electrical stimulation of the retina. An array of planar platinum stimulating electrodes was inserted into the suprachoroidal space of one eye in a normally sighted cat. Cortical activation was assessed by way of optical intrinsic signal (OIS) imaging. Cortical responses were recorded for three stimulus conditions, 1) visual stimulation alone, using oriented drifting square-wave gratings, 2) visual stimulation combined simultaneously with electrical stimulation, and 3) electrical stimulation alone. Electrical stimuli consisted of biphasic cathodic first current pulses (50Hz, 350µs per phase, 414µA). Images were acquired at 5Hz for a duration of 10s commencing 1s before stimulus onset. All stimuli in all conditions were 3s in duration. We observed significant differences in the modulation of cortical reflectance between the visual and visual-electrical conditions commencing ~3s after stimulus onset in a localised region of cortex ~1mm in diameter (t-tests, p < 0.05). Significant modulation of cortical reflectance was also observed in the same region of cortex and over a comparable time course following electrical stimulation alone. Our results are consistent with focal activation of the primary visual cortex. We propose that suprachoroidal stimulation represents a viable solution for implantation of a retinal prosthetic and that OIS imaging may be used to compare visual and electrically evoked responses in the visual cortex.

## POS-MON-185

## CANNABINOIDS MODIFY THE VISUAL SIGNAL IN THE RETINA

Middleton T.M.<sup>1,2</sup> and Protti D.P.<sup>1,2</sup><sup>1</sup>Bosch Institute, University of Sydney, NSW 2006. <sup>2</sup>Discipline of Physiology, School of Medical Sciences, University of Sydney, NSW 2006.

Endocannabinoids and their receptors have been localised to all retinal cells. The endocannabinoid system plays an important role in short term plasticity of excitatory and inhibitory synaptic activity in the CNS. Upon depolarisation of postsynaptic neurones, cannabinoids are synthesized on demand and retrogradely travel to activate presynaptic cannabinoid receptors (CB1R), which in turn reduce neurotransmitter release. These mechanisms modulate neuronal excitability and are likely disrupted by the addition of exogenous cannabinoids. The physiological role of the endocannabinoid system in the retina, however, is still unknown.

**Purpose:** To investigate the effects of cannabinoids on light responses in Retinal ganglion cells (RGCs) **Methods:** Whole cell patch clamp recordings from dark adapted mouse RGCs were carried out in the whole mount preparation. Response to light spots of varying size and contrast were recorded before and after the administration of the CB1 cannabinoid receptor agonist WIN55212-2 (5µM). **Results:** Overall, the addition of WIN55212-2 reduced the magnitude of light-responses. In 4 out of 5 ON cells and 3 out of 3 OFF, WIN55212-2 reduced the strength of the peak light-response, quantified as spikes and membrane potential, and in most cases decreased the inhibitory effect of surround stimulation. WIN55212-2 produced a dampening of light-responses at all contrast levels tested, causing a reduction in depolarisation in response to preferred contrast as well as a reduction in hyperpolarisation in response to non preferred contrast in 5 out of 6 ON cells and 3 out of 3 OFF cells. **Conclusion:** Our data demonstrates that exogenous cannabinoids modify the inputs into RGCs, thus altering the response to light. These results suggest that the endocannabinoid system modulates neuronal excitability in the retina.

## POS-MON-187

## INTRINSIC PHYSIOLOGICAL PROPERTIES OF RAT RETINAL GANGLION CELLS

Wong R.C.S., Sunder Raj D., Cloherty S.L., Ibbotson M.R. and O'Brien B.J.

Visual Sciences Group, Department of Psychology and ARC Centre of Excellence in Vision Science, Australian National University, Canberra, Australia.

Visual information is encoded by the retina through the complex synaptic arrangement of neurons within the retinal network. In addition to network processing, individual retinal neurons also have unique physiological properties which enhance their computational capacity. Ultimately, it is the intrinsic physiological properties of retinal ganglion cells (RGCs) which define how information is finally encoded and sent to brain nuclei. Most previous studies of rat RGC biophysics have focussed upon the properties of individual types of channels. We have now made whole-cell current clamp recordings of the intrinsic physiological properties of rat RGCs in retinal wholemounts maintained *in vitro*. Recordings were made from several different rat RGC types as identified by confocal reconstruction and morphological classification (Sun et al, 200x). These recordings have yielded a large amount of variability amongst the sample of cells recorded (n = 30). Passive membrane properties (mean, range): Resting membrane potential (-57.87 mV, [-47.34, -74.86] mV), time constant (16.29 ms, [10.71, 24.31] ms), input resistance (360.68 MΩ, [101.03, 795.48] MΩ). Spiking properties (mean, range): spike width (2.41 ms, [1.00, 4.96] ms), maximum frequency (112.30 Hz, [37.42, 245.89] Hz), steady state frequency (44.99 Hz, [15.55, 94.07] Hz), frequency adaptation index (0.54, [0.24, 0.9]). In addition, 6 of 30 cells exhibited anomalous rectification. This large amount of variability suggests that the processing of synaptic inputs into trains of action potentials is quite different among individual types of RGCs. It will be interesting to determine whether the spiking properties of individual RGC types are tuned to the synaptic properties of their central targets.

## POS-MON-186

## THE EFFECTS OF DRUG-SIMULATION ON THE RESPONSES OF RETINAL GANGLION CELLS OF ADULT MICE

Huang J.Y. and Protti D.A.

Disciplines of Biomedical Science and Physiology, School of Medical Sciences and Bosch Institute, University of Sydney, NSW 2006.

Retinal ganglion cells (RGCs) receive excitatory and inhibitory inputs from specific neuronal circuits formed by bipolar and amacrine cells respectively. The relative magnitude and timing of these inputs determine the spatial and temporal properties of RGCs. The relative impact of excitation and inhibition on RGC output, however, is difficult to evaluate as the pharmacological blockers used to identify and isolate these inputs not only act on the RGC recorded from but also in the whole retinal network. **Purpose:** To investigate the role of direct inhibitory input and presynaptic inhibition on spatial tuning properties of RGCs. **Methods:** Dynamic-clamp recordings were made from the cell bodies of RGCs in whole-mounts. Light-evoked synaptic conductances recorded in response to increasing spot diameters in control and under TTX were injected into RGCs. **Results:** We recorded from A, B and C RGCs subtypes (n=9). Injection of control conductances generated responses that peaked for small spots (150µm) and then decreased for larger spots (1400µm), consistent with the typical centre-surround organisation of receptive fields. Injection of conductances measured under TTX produced overall stronger responses, decreased surround inhibition and a small shift in the peak of tuning curves. Blockade of both direct inhibitory input and presynaptic inhibition by TTX produced the strongest effect, enhancing peak response and relieving surround inhibition. Removal of only presynaptic inhibition showed the second strongest effect for both parameters and lastly removal of direct inhibition showed smaller effects. **Conclusion:** Our data indicate that both direct inhibitory input and presynaptic inhibition contribute to the sharpening of spatial tuning in RGCs.

## POS-MON-188

## TTX-RESISTANT VOLTAGE GATED SODIUM CURRENTS ARE EXPRESSED IN MOUSE RETINAL GANGLION CELLS

Park S.J.H.<sup>1</sup> and O'Brien B.J.<sup>1,2</sup><sup>1</sup>Optometry & Vision Science, University of Auckland. <sup>2</sup>Visual Sciences Group & Dept. of Psychology, Australian National University.

Visual information is encoded by the retina through a complex interaction of neurons within the retinal network. In addition to network processing, retinal neurons also have unique biophysical properties which enhance their computational capacity. Recently, we have anatomically localised the tetrodotoxin-resistant Na<sub>v</sub>1.8 subunit of the voltage gated sodium channel family to retinal ganglion cells (RGCs). We therefore sought to determine whether TTX-R sodium currents are expressed in RGCs, and whether such sodium conductance serves a specialised role in their function. Whole-cell patch clamp recordings were performed at room temperature (~22°C). To isolate Na<sup>+</sup> currents, voltage-gated K<sup>+</sup> & Ca<sup>2+</sup> currents were suppressed using a Cs<sup>+</sup> based internal solution with 20 mM TEA and 0.1mM CdCl<sub>2</sub> in the perfusate. Addition of TTX (1µM) was used to determine whether TTX-R sodium currents were present. Retinal neurons were morphologically identified by inclusion of Lucifer yellow (0.5%) and Neurobiotin (0.05%) in the internal solution. Our data demonstrate that a subpopulation of RGCs express a TTX-R current that is activated near -65 mV and reaches a maximum amplitude of -340 pA at -50 mV (n = 12). Considerable overlap between activation and inactivation curves suggests this current could be a 'window' current acting near resting membrane potentials. TTX-R inward currents were demonstrated to be sodium mediated through ion substitution experiments. These results demonstrate, for the first time, the presence of TTX-R voltage-gated sodium currents in the retina. The presence of a potential window current suggests this channel may play a role in enhancing the excitability in RGCs through a form of persistent activation.

## POS-MON-189

## SPATIAL TUNING PROPERTIES OF EXCITATORY AND INHIBITORY SYNAPTIC INPUTS ONTO PRIMATE RETINAL GANGLION CELLS

Protti D.A., Vonhoff C.R., Di Marco S. and Solomon S.G.  
Discipline of Physiology and Bosch Institute, University of Sydney, NSW 2006.

Inner retinal inhibition is often thought to shape the temporal properties of the light response but not spatial tuning. **Purpose:** To determine the contribution of the inner retina to spatial tuning of retinal ganglion cells (RGCs) we measured the spatial organisation of excitatory and inhibitory inputs onto RGCs in the marmoset (*Callithrix jacchus*) retina. **Methods:** Voltage-clamp recordings were obtained from RGCs in whole-mount retinas in whole-cell mode. Light-evoked currents were measured in response to spots of different diameter; excitation and inhibition were isolated by clamping cells at -55 and 0 mV respectively. **Results:** Light increments elicited excitation and inhibition in all ON-RGCs ( $n=14$ ). Excitatory inputs were spatially tuned (14/14 cells); in 7/14 cells inhibitory inputs were tuned whilst the remaining cells displayed spatial summation. Light decrements elicited excitation in 14/16 OFF-RGCs but inhibition in only 3/16 cells; excitation was always size tuned. Two OFF-RGCs displayed a large tonic inhibitory input that was reduced by light decrements. Most ON and OFF cells showed strong inhibition for anti-preferred contrast steps (decrements and increments respectively). GABA<sub>A</sub> receptor antagonists reduced direct inhibitory inputs in all 10 RGCs tested (6 ON and 4 OFF) and decreased presynaptic surround inhibition. The voltage-gated sodium channel blocker TTX reduced direct inhibition and size-tuning of excitatory currents, indicating that tuning of excitatory inputs is at least partly due to inner retinal inhibition. **Conclusion:** Spatial tuning of excitatory inputs onto ON and OFF RGCs is similar whilst inhibitory inputs are asymmetric. Inner retinal inhibition shapes the spatial tuning of excitatory inputs of both ON and OFF RGCs and provides direct inhibitory input onto RGCs.

## POS-MON-190

## MODELLING OF ON AND OFF RETINAL GANGLION CELLS (RGCS)

Kameneva T.<sup>1</sup>, Meffin M.<sup>1</sup> and Burkitt A.N.<sup>2</sup>

<sup>1</sup>National ICT Australia. <sup>2</sup>The University of Melbourne, Electrical and Electronic Engineering, Parkville 3010 Australia.

ON and OFF RGCs have different patterns of firing in response to current clamp stimuli and generate maintained activity through different mechanisms (Margolis & Detwiler 2007). ON cells depend on tonic excitatory input to drive maintained activity. OFF cells maintain activity in the absence of synaptic input and exhibit subthreshold oscillations, rebound excitation, burst firing. Ionic channels mechanisms underlying these differences are not completely understood. Numerical simulations of single-compartment Hodgkin-Huxley type neurons were carried out in NEURON to investigate the role of hyperpolarisation activated,  $I_h$ , and low voltage activated  $Ca$ ,  $I_T$ , currents. Model parameters were constrained by fitting to the following experimental observables: (i) resting membrane potential (ON: -65mV; OFF: -55mV), (ii) spontaneous activity (ON: 0Hz; OFF Transient 19Hz; OFF Sustained 44Hz), (iii) pattern of the coefficient of variation of inter-spike-interval in OFF cells, (iv) presence or absence of subthreshold oscillations, rebound excitation, burst firing. All parameters for ON and OFF cells were set equal except for the maximum conductances of  $I_h$  and  $I_T$ ,  $g_h/g_T$ . A search of the parameter space for  $g_h$  and  $g_T$  was undertaken by variable iteration step (minimum step  $10^{-6}$  S/cm<sup>2</sup>) from  $g_h=g_T=0$  to  $g_h=g_T=0.1$  S/cm<sup>2</sup>. Two distinct sets of parameters were found that correspond to ON and OFF RGCs. The comparison of the experimental data with numerical simulations gave the following bounds upon the conductances: ON cells:  $g_h \leq 2.8 \times 10^{-5}$  S/cm<sup>2</sup>,  $g_T \leq 10^{-5}$  S/cm<sup>2</sup>; OFF cells:  $3.5 \times 10^{-4} \leq g_h \leq 1.1 \times 10^{-3}$  S/cm<sup>2</sup>,  $g_T \leq 10^{-5}$  S/cm<sup>2</sup>. Simulations show that differences in magnitudes of  $I_h$  and  $I_T$  account for differences in intrinsic properties of ON and OFF RGCs and support the hypothesis that  $I_T$  plays the main role in differentiating firing patterns between ON and OFF RGCs under synaptic blockage.

## POS-MON-191

## MORPHOLOGICAL CLASSES OF RETINAL GANGLION CELLS IN THE PIGEON RETINA

Querubin A.<sup>1,2</sup>, O'Brien B.J.<sup>2,3</sup> and Bumsted O'Brien K.M.<sup>1,2</sup>

<sup>1</sup>ARC Centre of Excellence in Vision Science. <sup>2</sup>Research School of Biology, The Australian National University. <sup>3</sup>Dept. of Psychology, The Australian National University, Canberra, ACT, 2601.

In the pigeon, a dorsal region of high visual acuity, the area dorsalis in the red field (RF), correlates with high retinal ganglion cell (RGC) densities (Querubin et al., 2009). To determine whether the pigeon RGC array in the RF contains a type similar to midget RGC in primates - our basis for high visual acuity, we aimed to characterise RGCs in the RF. RGCs were labelled with Dil or DiO in pigeon retinal wholemount preparations ( $n=16$ ) using a Di-olistics approach with coated tungsten beads (BioRad PDS-1000/He System). Labelled RGCs were imaged using a confocal microscope. Soma size, dendritic field size, inner plexiform layer (IPL) stratification and eccentricity were measured and then RGCs were grouped based on these parameters. Seventeen RGC types were identified from 216 cells throughout the retina and grouped into two broad categories: wide-field (5 types,  $>65\mu\text{m}$  diameter) and narrow-field (12 types,  $<65\mu\text{m}$  diameter). The most common wide-field type (105 $\mu\text{m}$  diameter) was monostriated near the RGC layer. A wide-field bistratified type was present mainly in the peripheral retina. The most prevalent narrow-field type (44 $\mu\text{m}$  diameter) was monostriated in the proximal IPL. Other narrow-field types were mono-, bi-, or diffusely stratified. The smallest dendritic field diameter observed in our sample (a bistratified cell) was 14  $\mu\text{m}$ , approximately twice the size required to resolve 12cpd (behaviourally measured acuity for the area dorsalis, Rounsley & McFadden, 2005). Our data suggest that there are ~17 different types of pigeon RGCs. None of the cell types corresponded to the primate midget type, though the smallest filled cell was sufficient to mediate ~6cpd.

## POS-MON-192

## UNIFORMITY DETECTOR GANGLION CELLS IN RABBIT RETINA

Sivyer B.<sup>1,2</sup>, Taylor W.R.<sup>3</sup> and Vaney D.I.<sup>1,2</sup>

<sup>1</sup>ARC Centre of Excellence in Vision Science. <sup>2</sup>Queensland Brain Institute, The University of Queensland. <sup>3</sup>Casey Eye Institute, Oregon Health & Science University, Portland, OR.

Retinal ganglion cells convey information by increasing their firing in response to an optimal visual stimulus or 'trigger feature'. However, one class of ganglion cell responds to changes in the visual scene by decreasing its firing. These cells, termed uniformity detectors in the rabbit retina, are encountered only rarely and the synaptic mechanisms underlying their unusual responses have not been investigated. We have been able to target uniformity detectors with a high success rate in a whole mount preparation of the rabbit retina, which has enabled us to characterise the synaptic mechanisms that govern their unusual light responses. Intracellular injection of Neurobiotin revealed that the uniformity detectors have a distinctive bistratified dendritic morphology: they branch at both margins of the inner plexiform layer, in the ON and OFF sublaminae ( $n=20$ ). The uniformity detectors show tracer-coupling to a population of GABAergic amacrine cells that co-stratify with the ganglion cells in the ON sublamina. The maintained firing of uniformity detectors is transiently suppressed by bright or dark contrast. Patch-clamp recordings show that the action potentials arise within 'complex spikes', each comprising a burst of 2 or 3 Na<sup>+</sup> spikelets riding on top of a slower Ca<sup>2+</sup>-mediated depolarization. Both ON and OFF visual stimuli elicit only inhibitory synaptic input ( $n=21$ ), the immediate effect of which is to suppress the maintained firing. However, this inhibition also alters the properties of the resurgent spiking by increasing the amplitude of the spikelets within each burst, suggesting that this may increase the efficacy of spike propagation and transmission. This appears to be the first report of a retinal ganglion cell that (1) produces complex spikes and (2) receives negligible bipolar cell input.



## POS-MON-193

## ORIENTATION SELECTIVE CELLS IN THE LATERAL GENICULATE NUCLEUS OF MARMOSETS

Cheong S.K.<sup>1,2</sup>, Lim J.K.H.<sup>1,2</sup>, Tailby C.<sup>1,2</sup>, Solomon S.G.<sup>3</sup> and Martin P.R.<sup>1,2</sup>

<sup>1</sup>National Vision Research Institute of Australia. <sup>2</sup>Dept. Optometry and Vision Sciences, The University of Melbourne. <sup>3</sup>Medical Sciences, The University of Sydney.

**Purpose:** Cells in the dorsal lateral geniculate nucleus (dLGN) are normally characterised as having receptive fields with circular concentric centre-surround structure. Orientation selectivity is considered to arise in the primary visual cortex (V1). Here we describe dLGN cells showing strong orientation selectivity. **Methods:** Single electrode, extracellular recordings were made in sufentanil-anaesthetised marmosets (*Callithrix jacchus*, n=7). Receptive fields were characterized using drifting sinusoidal gratings and orientation selectivity indices<sup>1</sup> (OSI) were calculated at close to or optimum spatial frequency (SF). Recordings were targeted to the koniocellular layers. **Results:** Eight putative koniocellular cells showed strong orientation selectivity (OSI $\geq$ 0.40, "o-cells") similar to V1 cells<sup>2</sup>. Where tested (n=4), three cells showed no response to s-cone modulation ("non-blue") and one cell showed weak s-cone response. The distribution of OSI values for other non-blue koniocellular cells was 0.085 $\pm$ 0.07 (mean $\pm$ S.D., n=61). Four o-cells showed higher selectivity for f0 than for f1 harmonic. Where tested (n=2), o-cells responded to stimulation in both eyes, however, one eye was dominant. Optimum SF (mean $\pm$ range: 0.96 $\pm$ 1.47 cycles/deg, n=7) was similar to other koniocellular cells<sup>2</sup>. Relative low SF response ratios (mean $\pm$ range: 0.22 $\pm$ 0.36, n=7) indicated band-pass SF tuning. Contrast tuning was linear where tested (n=5). Extracellular recording waveforms were consistent with soma recording. **Conclusions:** A subpopulation of putative koniocellular cells shows strong orientation selectivity. The selectivity could originate in the retina, the visual cortex, or the superior colliculus. All these areas project to the koniocellular layers. **References:** 1. Levick WR, Thibos LN (1982) J. Physiol. 329:243-261. 2. Forte JD, Hashemi-Nezhad M, Dobbie WJ, Dreher B, Martin PR (2005) Vis. Neurosci 22:479-491.

## POS-MON-195

## LOCAL MOTION DETECTION: TEMPORAL AND SPATIAL MODULATION OF GAIN AND TRANSIENT RESPONSES TO FEATURES IN NATURAL IMAGES

Barnett P.D.<sup>1</sup>, Nordström K.<sup>1,2</sup> and O'Carroll D.C.<sup>1</sup>

<sup>1</sup>Discipline of Physiology, The University of Adelaide, SA, 5005 Australia. <sup>2</sup>Department of Neuroscience, Uppsala University Biomedical Centre, Box 593, 75124 Uppsala, Sweden.

Navigating within the natural environment is a challenging task for visual systems. Natural scenes vary enormously in brightness, color, and contrast. Yet many animals adopt visually guided behavior for which the accurate interpretation of motion is required. Recently, HSN and HSNE neurons have been identified in the hoverfly, which accurately encode the velocity of image motion to natural scenes. Natural scenes produce highly variable local responses from such neurons, yet their global responses are highly reliable between images, a property that we hypothesize derives from local adaptation within the scene. We recorded intracellularly from HSN & HSNE neurons (n>10 for each neuron class) to investigate how motion coding is shaped by local adaptation of transient responses to passing features. Stimuli were displayed either globally, across the whole receptive field, or limited to a small patch in the receptive field. We show that local adaptation is contrast dependent, which leads to differences in transient responses depending on the order of local contrasts experienced. When low contrast features pass a location within the receptive field, they exert little effect on subsequent responses, but even transient stimuli with high contrasts lead to potent suppression of the response to subsequent features. We show that this effect is facilitated by simultaneous activity of neighboring local motion sensitive elements perpendicular to the direction of image motion. Local modulation of response gain based on activity of the surrounding area is ideally suited to take advantage of the statistically predictable nature of natural scenes.

## POS-MON-194

## CONTRIBUTION OF WIDE-FIELD GANGLION CELLS TO CENTRAL VISION IN PRIMATE RETINA

Percival K.A.<sup>1,2</sup>, Martin P.R.<sup>1,2</sup> and Grunert U.<sup>1,2</sup>

<sup>1</sup>Department of Optometry & Vision Sciences, The University of Melbourne, Carlton VIC 3053. <sup>2</sup>National Vision Research Institute, Carlton, VIC 3053.

**Purpose:** The contribution of non-midget, non-parasol (or wide-field) retinal circuits to foveal vision is poorly understood. Here we investigated wide-field ganglion cells and diffuse type bipolar cells in central retina. **Methods:** Ganglion cells were retrogradely labeled by tracer injections to the posterior koniocellular layers of marmoset (*Callithrix jacchus*) lateral geniculate nucleus, and subsequently photofilled. Bipolar cell types DB3, DB4 and DB6 were labeled immunohistochemically in vertical sections of macaque (*Macaca fascicularis*) retina through the foveal pit, and identified according to stratification of axon terminals (Chan et al., 2001). **Results:** Of the 37 labeled wide-field ganglion cells found within 1 mm from the fovea, nine were small bistratified (blue-ON/yellow-OFF) ganglion cells, six were large sparse ganglion cells and one was a broad thorny type similar to those described in the periphery (Dacey et al., 2003; Szmajda et al., 2008). The remaining cells included a bistratified type (n = 3) with sparsely branching dendrites in the ON and OFF sublamina of the inner plexiform layer, cells with narrowly stratified dendrites (n = 6 stratifying in the ON-, n = 5 stratifying in the OFF sublamina) and 6 cells with broadly stratified dendrites. A high proportion of small bistratified and large sparse types was also found in peripheral retina (Szmajda et al., 2008). All cone bipolar cell types investigated were present within 1 mm from the foveal pit. **Conclusion:** Wide-field ganglion cells, and the diffuse bipolar cell types which likely provide their input, are present in central retina and could potentially contribute to vision at the fovea.

## POS-MON-196

## HONEYBEE NEUROBIOLOGY- MOVEMENT DETECTION IN THE HONEYBEE (APIS MELLIFERA)

Hung Y.S., Van Kleef J., Gert S. and Ibbotson M.

Visual Sciences Group and ARC Centre of Excellence in Vision Science, Australian National University, Canberra, Australia.

The honeybee motion-sensitive interneurons descending from the brain to the thoracic motor centers have been morphologically identified, and the electrophysiological responses of these cells to motion stimuli have also been verified in previous studies. However, the spectral characteristics of these cells remain unknown. From behavioral experiments, it is thought that the optomotor responses in bees are achromatic and exclusively driven by green photoreceptors. In this study, intracellular electrophysiology experiments were carried out on honeybee motion-sensitive descending neurons. The results show that the motion-sensitive descending neurons are UV-sensitive. Despite the fact that the green photoreceptors have weak spectral sensitivities into the UV region of the spectrum, the cells showed strong responses to UV stimuli. Cell responses with and without ocellar inputs were recorded to verify the visual inputs for the motion-sensitive descending neurons. With the ocellar input, responses were characterized by an excitatory rebound for the anti-preferred direction. This suggests that the motion-sensitive neurons respond to signals from both the compound eyes and the ocelli.

## POS-MON-197

**FEATURE-DETECTING NEURONS IN THE DRAGONFLY AND THEIR ELECTROPHYSIOLOGICAL RESPONSES TO NATURAL STIMULI**

Wiederman S.D. and O'Carroll D.C.

Discipline of Physiology, The University of Adelaide, SA, Australia.

Intracellular recordings from identified neurons within the optic lobe of the dragonfly (*Hemicordulia tau*) reveal selectivity for small moving objects<sup>1</sup>. These 'hypercomplex' neurons, referred to as small target motion detectors (STMDs), may contribute to the visual discrimination of moving features (e.g. prey, predators and conspecifics) against complex, moving backgrounds. We have modeled properties of one such neuron, CSTMD1<sup>2</sup> and run simulations that predict the responsiveness of this neuron to a series of natural image stimuli. These model runs indicate the location of a *rare* set of 'false positive' (target-like) features within each of the scenes. We validated these model predictions by recording intracellularly from the CSTMD1 neuron in an immobilized dragonfly, whilst displaying rotating, panoramic images (5 dragonflies, 6 images, average total of 44 repeats for each image). Recent experiments<sup>3</sup> with a 'two target' stimulus paradigm showed that CSTMD1 includes long-range and inter-hemispheric inhibitory interactions. We present an extended modeling effort that includes these complex, receptive-field properties and aids in the interpretation of response characteristics. Additionally, we obtained further electrophysiological recordings whilst varying the natural image parameter space; panorama velocity, position, contrast and image extent (within the receptive field subregions of CSTMD1). These electrophysiological results, in conjunction with the modeling, help elucidate both the mechanisms and possible roles of CSTMD1 in the detection and pursuit of moving targets. [1] O'Carroll (1993) Nature 362, 6240, 541 [2] Wiederman et al. (2008) PLoS ONE, 3, 7, e2784 [3] Bolzon et al. (2009) J Neurosci (in press).

## POS-MON-198

**POST-SYNAPTIC GABA<sub>A</sub> RECEPTOR NUMBERS ARE REDUCED IN PURKINJE CELLS OF THE DYSTROPHIN-DEFICIENT mdx MOUSE**Kueh S.L.L.<sup>1,2</sup>, Head S.I.<sup>1</sup> and Morley J.W.<sup>1,2</sup><sup>1</sup>School of Medical Sciences, University of New South Wales. <sup>2</sup>School of Medicine, University of Western Sydney.

Duchenne muscular dystrophy (DMD) is caused by the absence of the protein dystrophin. DMD is characterized by progressive muscle weakness, loss of skeletal muscle fibres and premature death. Around a third of DMD boys also present with an accompanying cognitive impairment. In the cerebellum, dystrophin is localized at the postsynaptic membrane of GABAergic synapses on Purkinje cells. Utilising the *mdx* mouse model of DMD we have previously shown an enhanced tonic inhibition in *mdx* mice and hypothesize that this is due to the increase in extrasynaptic GABA<sub>A</sub> receptors which occurs as a direct consequence of the absence of dystrophin from the post-synaptic density. In the present study we have looked at the effect of an absence of dystrophin on the number and function of GABA<sub>A</sub> receptor channels located at the post synaptic density. Whole-cell patch-clamp recordings of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were performed in cerebellar slices from *mdx* and littermate control mice. Immunofluorescence assays were performed on fresh frozen section. Using non stationary noise analysis we found a significant reduction in the number of receptors at GABAergic synapses in *mdx* mice ( $38.38 \pm 2.95$ ;  $n=14$ ) compared to littermate controls ( $53.03 \pm 4.11$ ;  $n=12$ ) ( $p=0.01$ ). These electrophysiological findings were supported by the immunofluorescent assay, which showed a reduced density of GABA<sub>A</sub> receptors in the post synaptic density. The expression of GAD-6 and Gephyrin was unchanged in the *mdx* mice compared to littermate control. Our results demonstrate that dystrophin plays a role in ion channel localization in the CNS.

## POS-MON-199

**GABA-B RECEPTORS REGULATE THE EXCITABILITY OF CORTICAL LAYER 5 PYRAMIDAL NEURONS VIA LOCATION-DEPENDENT MECHANISMS**

Breton J.D. and Stuart G.J.

Neuroscience Program / The John Curtin School of Medical Research / Australian National University.

GABAergic inhibition in the neocortex is mediated by either GABA-A (ionotropic) or GABA-B (metabotropic) receptors. Activation of GABA-B receptors typically is thought to open G protein-coupled inwardly rectifying potassium (GIRK) channels and can also down-regulate voltage-activated calcium channels. Postsynaptic GABA-B receptors play a role in setting the resting membrane excitability, whereas presynaptic GABA-B receptors can regulate transmitter release. Here we investigated the impact of GABA-B receptor activation on the excitability of layer 5 pyramidal neurons in brain slices of rat barrel cortex. At the soma, GABA-B receptor activation via bath application of baclofen (20  $\mu$ M) was associated with hyperpolarization of the resting membrane potential and a decrease in input resistance, leading to a strong and reversible decrease in the number of action potentials evoked by somatic current injections ( $n=41$ ,  $P<0.001$ ). Surprisingly, these somatic effects of baclofen were not blocked by the GIRK channel antagonist tertiapin (100 nM). In contrast, GABA-B receptor activation did not affect the dendritic resting membrane potential, input resistance or somatodendritic steady-state voltage attenuation ( $n=7$ ,  $P>0.05$ ). To confirm a differential contribution of GABA-B receptor activation on somatic and dendritic resting membrane excitability, we locally applied baclofen (50  $\mu$ M) to the soma and distal dendrites. No effect of baclofen was observed at distal dendritic sites ( $n=3$ ). Despite the absence of dendritic GABA-B receptor activation on membrane excitability, bath application of baclofen blocked dendritic calcium electrogenesis evoked by high frequency action potential trains ( $n=17$ ). These data suggest that GABA-B receptors regulate the somatic and dendritic excitability of cortical layer 5 pyramidal neurons via different and location-dependent mechanisms.

## POS-MON-200

**LIGAND-INDUCED CONFORMATIONAL CHANGES IN THE  $\alpha 1\beta 2\gamma 2$  GABA-A RECEPTOR PROBED USING VOLTAGE-CLAMP FLUOROMETRY**Wang Q.<sup>1</sup>, Pless S.A.<sup>2</sup> and Lynch J.W.<sup>1</sup><sup>1</sup>Queensland Brain Institute, University of Queensland. <sup>2</sup>School of Biomedical Sciences, University of Queensland.

GABA-A chloride channel receptors mediate most inhibitory neurotransmission in the central nervous system. To date there is little information on the conformational changes induced by different ligands in different subunits. The loop F of  $\alpha 1$  subunit ligand-binding domain forms part of the GABA binding site, and previous studies predicted this domain might be involved in channel activation. The M2-M3 linker is an important component of the channel opening mechanism. We used voltage-clamp fluorometry to monitor the conformational changes induced by different agonists and antagonists in loop F of the  $\alpha 1$ ,  $\beta 2$  and  $\gamma 2$  subunits, and in the M2-M3 linkers of the  $\alpha 1$  and  $\beta 2$  subunits. We generated the  $\alpha 1$ -R186C,  $\alpha 1$ -N275C,  $\beta 2$ -I205C,  $\beta 2$ -K298C and  $\gamma 2$ -S195C mutations. GABA-A receptors comprising mutated  $\alpha 1/\beta 2$  and  $\alpha 1/\beta 2/\gamma 2$  subunits were then recombinantly expressed in *Xenopus* oocytes. These receptors were studied using simultaneous voltage-clamp and micro-fluorometry. We successfully labeled  $\alpha 1$ -R186C,  $\beta 2$ -K298C,  $\alpha 1$ -N275C,  $\gamma 2$ -S195C and  $\beta 2$ -I205C with sulfhydryl-reactive rhodamine derivatives. We then monitored the fluorescence change induced by different agonists (GABA and  $\beta$ -alanine), a competitive antagonist (SR-95531) and an allosteric modulator (diazepam). Agonists and antagonists induced similar conformational changes in loop F of  $\alpha 1$  subunit, whereas they evoked the different conformational changes in loop F of the  $\beta 2$  subunit (antagonist induced a  $52\% \pm 9.8\%$  smaller fluorescence change than GABA induced ( $n>4$ ,  $p<0.01$ ). In loop F of the  $\gamma 2$  subunit we observed fluorescence changes induced by GABA and diazepam ( $n>4$ ). The M2-M3 linkers of both the  $\alpha 1$  and  $\beta 2$  subunits produced different fluorescence changes with agonists and antagonist ( $n>4$ ). From our preliminary study,  $\alpha 1$ -R186C and  $\beta 2$ -I205C were not involved in the diazepam-induced conformation change. The results suggest that different GABA receptor subunits respond differently to the binding of agonists and antagonists.

## POS-MON-201

**LOCATION OF NR2B SUBUNIT-CONTAINING NMDA RECEPTORS AND THEIR CONTRIBUTION TO DIFFERENT FORMS OF LTP**

**Lohmann P.**, Johnstone V.P.A. and Raymond C.R.  
Neuroscience Program, The John Curtin School of Medical Research,  
The Australian National University, Canberra.

Long-term potentiation (LTP) is a diversified phenomenon. In area CA1 of the hippocampus, varied forms of LTP have been shown to coexist, each involving different intracellular signalling and effector cascades. Most forms of LTP are dependent on activation of postsynaptic NMDARs, however controversy exists over the relative roles of receptors containing different NR2 subunits. We have investigated the involvement of NMDARs containing the NR2B subunit in different forms of LTP at the CA3-CA1 synapses in hippocampal slices from male Wistar rats (8-9wks). The selective NR2B antagonist RO 25-6981 (1 $\mu$ M) had no effect on short- and long-lasting LTP induced by 1 and 8 trains of theta-burst stimulation, respectively (1TBS, n=6; 8TBS, n=6) but dramatically reduced the magnitude and persistence of an intermediate LTP induced by 4 TBS (n=10, p<0.01). To assess the location of NR2B-containing NMDARs isolated NMDA fEPSPs were recorded and glutamate spill-over was enhanced by delivering a 5-pulse burst at 100Hz. RO 25-6981 (1 $\mu$ M) had no effect on these synaptic burst responses (n=4). However, after inhibiting synaptic NMDARs with the use-dependent channel blocker MK-801 (10 $\mu$ M) RO 25-6981 significantly reduced burst-induced response (n=4, p<0.01). Together these data show that an intermediate form of LTP but not short- and long-lasting LTP requires the activation of NR2B subunit-containing NMDARs and that these receptors are predominantly located extrasynaptically.

## POS-MON-203

**GROUP I METABOTROPIC GLUTAMATE RECEPTORS TONICALLY REGULATE SYNAPTIC GABA<sub>A</sub> RECEPTOR FUNCTION IN MIDBRAIN NEURONS**

**Drew G.M.**, Mitchell V.M. and Vaughan C.W.  
Pain Management Research Institute, Kolling Institute, University of  
Sydney at Royal North Shore Hospital, NSW, Australia.

GABA<sub>A</sub> receptors mediate the principal form of fast synaptic inhibition in the brain. Although phosphorylation of GABA<sub>A</sub> receptors has been demonstrated to alter receptor function in a number of in vitro expression systems, the physiological conditions under which native neurons undergo such modulation are largely unknown. Here, we examined the interaction between group I metabotropic glutamate receptors (mGluRs) and GABA<sub>A</sub> receptors using whole-cell patch-clamp recordings of periaqueductal grey (PAG) neurons in rat midbrain slices. We found that endogenous activation of group I mGluRs by the glutamate transporter inhibitor TBOA (n=3-8) produced a concentration-dependent reduction in GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic current (IPSC) decay times. A similar effect was observed following direct activation of group I mGluRs by the selective agonist DHPG (n=8). DHPG-induced shortening of IPSC decay was abolished by addition of the G-protein inhibitor GDP- $\beta$ S to the patch pipette (n=4) and mimicked/occluded by substituting physiological cations in the patch pipette for Tris<sup>+</sup> (n=5) to selectively impair cation-dependent glutamate uptake into the recorded neuron. Conversely, the mGluR1-specific antagonist CPCCOEt (n=13) and the mGluR5-specific antagonist MPEP (n=8) both significantly slowed IPSC rise and decay times. These results indicate that synaptic GABA<sub>A</sub> receptor function in PAG neurons is tonically controlled by postsynaptic group I mGluR activation and may provide a novel functional role for group I mGluRs localised within the postsynaptic specialisations of midbrain GABAergic synapses.

## POS-MON-202

**EFFECTS OF KA-INDUCED SEIZURE ON EGFP INTERNEURONS AND nNOS EXPRESSION IN THE GIN MICE CA3 REGION**

**Mohd Ali S.H.**<sup>1</sup>, Cosgrave A.S.<sup>2</sup> and Thippeswamy T.<sup>2</sup>  
<sup>1</sup>Universiti Sains Islam Malaysia, Malaysia. <sup>2</sup>University of Liverpool, UK.

**BACKGROUND:** Kainic acid (KA) is widely used in epileptogenesis study and nitric oxide (NO) is linked in KA-induced seizure. Hippocampal CA3 region is implicated in epileptogenesis. Transgenic mice expressing enhanced-GFP (GIN mice) in a subpopulation of GABAergic interneurons (EGFP interneurons) are valuable for epileptogenesis study as changes in these interneurons can be evaluated without further staining. **AIMS:** To assess the effects of KA-induced seizure on EGFP interneurons and nNOS expression in the hippocampal CA3 region of the GIN mice. **METHOD:** Twenty-two juvenile adult GIN mice were pre-treated with intraperitoneal NG-nitro-L-arginine methylester (L-NAME; 50 mg/kg) or normal saline twice daily for two days. Then, some mice were given KA (35 mg/kg, i.p), the rest given normal saline. Study involved four groups (n=3): CONTROL, L-NAME, KA, KA+L-NAME. After KA (2-hour study), animals were observed for two hours for seizure behaviour (Racine scale 1972), then sacrificed (pentobarbitone; 80 mg/kg). In the 24-hour study, diazepam was administered to all KA-treated mice after seizure onset, further observed for 24 hours and sacrificed. Brains were processed for nNOS enzyme immunohistochemistry. All procedures were performed under UK Home Office regulations. **RESULT:** EGFP interneurons were reduced in KA (p<0.05) and KA+L-NAME (p<0.01) groups in the 2-hour study, no significant effects observed in the 24-hour study. nNOS expression was increased in KA (p<0.001) and KA+L-NAME (p<0.001) groups in the 2-hour study and in KA group (p<0.001) in the 24-hour study following KA. **CONCLUSION:** KA-induced seizure reduces the number of EGFP interneurons and increases nNOS expression in the hippocampal CA3 region of the GIN mice. NO may have differential effects on the EGFP interneurons and may also modulate seizures.

## POS-MON-204

**ANGIOTENSIN II (ANG II) DECREASES GLUTAMATERGIC SYNAPTIC TRANSMISSION IN RAT SUPERFICIAL MEDULLARY DORSAL HORN**

**Rohampour K.**<sup>1,3</sup>, Williams M.<sup>1</sup>, Allen A.M.<sup>2</sup> and Jennings E.A.<sup>1</sup>  
<sup>1</sup>Department of Anatomy and Cell Biology, University of Melbourne, Melbourne, Australia. <sup>2</sup>Department of Physiology, University of Melbourne, Melbourne, Australia. <sup>3</sup>Department of Physiology, Tarbiat Modares University, Tehran, Iran.

**Introduction:** Clinical trials indicate that angiotensin-converting enzyme inhibitors are effective in the prophylactic treatment of migraine, and that this does not involve action on blood pressure. Nociceptive information from migraine headache is conveyed centrally via trigeminal primary afferent neurons and angiotensin AT1 receptors are located on these neurons, but their function remains unclear. **Methods:** We used whole-cell patch clamp physiology to study the effects of Ang II on miniature excitatory postsynaptic currents (mEPSCs) in spinal trigeminal substantia gelatinosa (SG- also called medullary dorsal horn) neurons in Sprague Dawley pups. **Results:** The mean ( $\pm$ SEM) mEPSC rate was 6.1  $\pm$  0.9. In control conditions superfusion of Ang II (1 $\mu$ M) caused a significant decrease (34%) in mEPSC rate to 4.0  $\pm$  0.8 (n=18, P<0.01 paired t-test), without affecting the mEPSC amplitude. In 5 of these neurons addition of the AT1 receptor antagonist, Candestaran (1 $\mu$ M), caused a reversal of the Ang II induced decrease in mEPSC rate (to 91% of baseline). **Conclusion:** These data suggest that activation of presynaptic AT1 receptors causes a decrease in glutamate release from trigeminal afferent terminals.



## POS-MON-205

**N-ARACHIDONYL-GLYCINE INHIBITS GLYCINE TRANSPORT IN RAT SUPERFICIAL DORSAL HORN**Jeong H.-J.<sup>1</sup>, Vandenberg R.J.<sup>2</sup> and Vaughan C.W.<sup>1</sup><sup>1</sup>Pain Management Research Institute, Kolling Institute of Medical Research, Northern Clinical School University of Sydney at Royal North Shore Hospital, New South Wales, Australia. <sup>2</sup>Department of Pharmacology, Bosch Institute, University of Sydney, New South Wales, Australia.

The arachidonyl amino acid N-arachidonyl glycine (NAGly) is expressed at high levels within the spinal cord and produces analgesia following spinal delivery, via mechanisms which differ to the related endocannabinoid arachidonyl ethanolamide (anandamide). It has recently been demonstrated that NAGly inhibits the cloned glycine transporter GLYT2. Here, we examined the actions of NAGly on neurons in lamina II of the superficial dorsal horn, a key site for the actions of many analgesic agents. NAGly prolonged the duration of GlyR-mediated currents induced by exogenous application of glycine, but not by  $\beta$ -alanine. NAGly and the GLYT2 inhibitor ALX-1393, but not the GLYT1 inhibitor ALX-5407 produced an inward current and an increase in noise which was abolished by strychnine. ALX-5407 and ALX-1393, but not NAGly prolonged the decay phase of GlyR-mediated spontaneous miniature IPSCs. By contrast, NAGly, ALX-5407 and ALX-1393 all prolonged the decay phase of GlyR-mediated evoked IPSCs. The effect of NAGly on evoked IPSCs was increased during rapid train stimulation. NAGly had no effect on IPSC rise-time, or amplitude. These findings suggest that NAGly enhances inhibitory glycinergic synaptic transmission within the superficial dorsal horn by blocking glycine uptake via a transporter, possibly GLYT2, which is located outside the glycine synaptic cleft.

## POS-MON-207

**INHIBITION IN THE LATERAL VESTIBULAR NUCLEUS**

Stitt I.M., Drury H., Ford D., Callister R.J., Brichta A.M. and Lim R. School of Biomedical Sciences and Pharmacy, The University of Newcastle.

The lateral vestibular nucleus (LVN) projects to all regions of spinal cord for innervation of axial and limb muscles to maintain posture and balance. The LVN consists predominantly of large Deiters neurons. Inhibition of Deiters neurons arises predominantly from cerebellar Purkinje cells and is GABAergic in origin. A recent study has shown a glycinergic projection from fastigial nucleus. This study investigates inhibition onto large Deiters neurons and interneurons of the LVN. **Immunofluorescence:** Mice (approx. 3 weeks old) were anaesthetised with Ketamine (100mg/kg) and transcardially perfused with saline, followed by 4% paraformaldehyde. Brains were removed and postfixed for 1 hour. Immunolabelling of GABA<sub>A</sub>, glycine receptors, and anchoring protein, gephyrin, showed immunofluorescence in LVN. **Electrophysiology:** Mice were anaesthetized as above and decapitated. Brains were removed and the region containing the LVN was sectioned (300  $\mu$ m). Approximately 73% of Deiters neurons are tonically active, and have comparable discharge rate (mean 9.69 Hz, n = 6) to nearby medial vestibular nucleus neurons (mean 9.71 Hz, n = 27). GABA<sub>A</sub>ergic and glycinergic mIPSCs were recorded in the presence of TTX (1  $\mu$ m) and CNQX (10  $\mu$ m) and their respective antagonists, strychnine (1  $\mu$ m) and bicuculline (10  $\mu$ m). Recordings from 45 neurons showed a differential inhibitory input to Deiters and interneurons. Deiters neurons received predominantly GABA<sub>A</sub>ergic inhibitory input, of very high frequency (mean frequency = 13.25 Hz, n=7), while interneurons received both GABA<sub>A</sub>ergic and glycinergic inputs. Preliminary results also show a rostrocaudal difference in the degree of GABA<sub>A</sub>ergic and glycinergic input onto Deiters neurons.

## POS-MON-206

**THE INTRA-CORTICAL ORIGIN OF ABSENCE-LIKE SEIZURES IN THE GAERS MODEL IS LOCATED IN THE SOMATOSENSORY S2 CORTEX**Zheng T.<sup>1,2</sup>, Morris M.J.<sup>3</sup>, Jovanovska V.<sup>1</sup>, Van Raay L.<sup>1</sup>, Gandrathi A.<sup>1</sup>, Reid C.A.<sup>4</sup>, O'Brien T.J.<sup>1</sup> and Pinault D.<sup>2</sup><sup>1</sup>Departments of Medicine, Surgery and Neurology, The Royal Melbourne Hospital, The University of Melbourne, Parkville, Victoria, AUSTRALIA. <sup>2</sup>INSERM U666, physiopathologie clinique et expérimentale de la schizophrénie, Université de Strasbourg (Faculté de Médecine), Strasbourg, France. <sup>3</sup>Department of Pharmacology, University of New South Wales, Kensington, NSW. <sup>4</sup>Centre for Neuroscience, The University of Melbourne.

**Introduction:** The intra-cortical localisation of the seizure generator in the Genetic Absence Epileptic Rats from Strasbourg (GAERS) is still unknown. This study localised and characterised the site of seizure initiation within the somatosensory cortex at the cellular and network level. **Methods:** Depth EEG recordings were performed in freely moving GAERS (n=6) and non-epileptic control rats (NECs, n=3). In a separate set of experiments, single-cell juxtacellular recordings of cortical neurons were made along with EEG recording of the related sensorimotor cortex *in vivo* under neurolept-anaesthesia in adult male GAERS (n=19) and NEC rats (n=5). **Results:** In freely moving animals, depth multi-site recordings revealed that seizures were initiated within the somatosensory S2 cortical region. The 5-9 Hz oscillations in S2 preceded the S1 by up to 3 seconds (n=6). Furthermore, typical SWD events were evoked by delivering a electrical stimulus train (7 Hz, 2 seconds) to the somatosensory cortical regions of the GAERS. A significantly smaller current was required to initiate SWD events in the S2 vs. the S1 Ulp region (mean $\pm$ s.e.m., 146 $\pm$ 31 $\mu$ A vs 257 $\pm$ 56 $\mu$ A, n=7, p=0.025). Stimulation train induced oscillations but not SWDs in the NEC rats (n=3). Juxtacellular recordings from both the GAERS and NECs revealed a population of cells within S2 and immediate adjacent cortical regions that fire rhythmically during both ictal and interictal periods at similar frequencies (6-15Hz, GAERS, 37 of 178 cells, 21%; NEC rats 13 of 78 cells, 17%). **Conclusions:** These results extend the "cortical theory" of absence seizures by localising the S2 region as the likely generator of SWD events. A population of inherently rhythmically firing cortical cells were identified in and around the S2 region. These cells may be acting as the initiators of the 5-9 Hz somatosensory rhythm which subsequently triggers absence seizures in epileptic animals.

## POS-MON-208

**EFFECTS OF SIMVASTATIN AND 6-HYDROXYDOPAMINE LESION ON HISTAMINERGIC H1 RECEPTOR BINDING IN RAT BRAINS**Hu C.H.<sup>1,2</sup>, Deng C.<sup>1</sup>, Huang X.-F.<sup>1</sup>, Chen J.<sup>1</sup> and Wang Q.<sup>1,3</sup><sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia. <sup>2</sup>School of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China. <sup>3</sup>Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, China.

Statins have been widely used for the treatment of a variety of medical conditions including neurological disorders beyond their original role in lowering cholesterol. The histamine receptors play an important role in neural regulation. However, it is yet unknown whether statins act on histamine receptors, particularly for their neuroprotective effects. **METHODS:** After pre-treatment with simvastatin (saline, or 1 or 10mg/kg/day, n=14-16) for 5 days, a half of each group were treated with 6-hydroxydopamine (6-OHDA) and the other half with sham-treatment, followed by 3-week treatments of simvastatin as mentioned above. Histamine H1 receptors (H1R) were detected by [<sup>3</sup>H]pyrilamine binding autoradiography. **RESULTS:** Compared to the saline group, simvastatin (1mg/kg/day) significantly decreased H1R bindings in the primary motor cortex (M1), ventromedial hypothalamic nucleus (VMH), caudate putamen (CPu), accumbens core (AcbC), prefrontal cortex (PF) (all p<0.05); however 10mg/kg/day simvastatin increased H1R density in the medial amygdaloid nucleus (p<0.05), but no significant effect in other regions detected. 6-OHDA lesion did not alter H1R binding density in most brain areas, except a decrease in the cingulate cortex (p=0.05). No interacted effect between simvastatin and 6-OHDA was observed. **CONCLUSION:** Simvastatin has different effects on the H1R in various brain regions of rats, which was not interacted with 6-OHDA lesion. These results suggest that simvastatin can modulate histaminergic neurotransmission in the brain, and support the role of H1 receptors in neurodegenerative disorders.

## POS-MON-209

### ACTIVATION OF $\alpha_1$ -ADRENERGIC RECEPTOR IN LAYER II/III PYRAMIDAL NEURONES IN SOMATOSENSORY CORTEX OF RAT CAUSES CALCIUM RELEASE FROM STORES

Choy J.<sup>1</sup> and Stricker C.<sup>1,2</sup>

<sup>1</sup>The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200. <sup>2</sup>ANU Medical School, The Australian National University, Canberra, ACT 0200.

We have previously shown that noradrenaline (NA) activates  $\alpha_1$ -ARs in barrel cortex. We now show that  $\alpha_1$ -AR activation causes  $\text{Ca}^{2+}$  release from presynaptic stores modulating transmitter release. **Purpose:** To characterise how  $\alpha_1$ -ARs cause  $\text{Ca}^{2+}$  mobilisation from presynaptic stores. **Methods:** 300  $\mu\text{m}$  thick parasagittal slices were prepared from P15-19 rats. Miniature excitatory postsynaptic currents (mEPSCs) were recorded from pyramidal cells in layer II/III, which were subsequently verified histologically. Voltage-clamp recordings were obtained at  $36\pm1^\circ\text{C}$  in the presence of tetrodotoxin (1  $\mu\text{M}$ ) and gabazine (3  $\mu\text{M}$ ).  $\alpha_1$ -ARs were activated by NA (10  $\mu\text{M}$ ) and  $\beta$ -ARs were blocked by propranolol (PO; 1  $\mu\text{M}$ ).  $\text{IP}_3$  receptors were blocked by 2-APB (16  $\mu\text{M}$ ). SERCA pump was blocked by cyclopiazonic acid (CPA; 20  $\mu\text{M}$ ) in conjunction with a brief  $\text{K}^+$  depolarisation to deplete the  $\text{Ca}^{2+}$  stores. Superfusion rate was 4 mL/min. **Results:** Co-application of PO (n=8) with NA showed a  $30\pm5\%$  increase in mEPSC frequency (from  $39\pm5$  to  $51\pm5$  Hz). With PO present, the increase in mEPSC frequency was sustained, whereas a transient increase occurred with NA alone. In the presence of 2-APB (n=9), mEPSC frequency decreased significantly by  $24\pm4\%$  ( $41\pm3$  to  $31\pm4$  Hz) and the subsequent NA application did not increase mEPSC frequency. When stores were depleted (CPA application plus  $\text{K}^+$  depolarisation; n=12), a significant drop of  $17\pm2\%$  in mEPSC frequency was observed ( $41\pm3$  to  $34\pm3$  Hz), which was not reversed by NA application. **Conclusions:** Presynaptic  $\alpha_1$ -ARs and  $\beta$ -ARs are present in rat somatosensory cortex and signalling via  $\alpha_1$ -ARs causes  $\text{IP}_3$  production leading to the activation of  $\text{Ca}^{2+}$  release from stores.

## POS-MON-211

### COMPARATIVE ELECTROPHYSIOLOGICAL PROPERTIES OF LOCUS COERULEUS NEURONS IN YOUNG AND ADULT MICE

de Oliveira R.B.<sup>1</sup>, Howlett M.C.<sup>1,2</sup>, Gravina F.S.<sup>1</sup>, Callister R.J.<sup>1</sup>, Brichta A.M.<sup>1</sup> and Van Helden D.F.<sup>1</sup>

<sup>1</sup>School of Biomedical Sciences, University of Newcastle, NSW Australia 2308. <sup>2</sup>Retinal Signal Processing, Netherlands Institute of Neuroscience, Amsterdam, The Netherlands 1105BA.

Neuronal plasticity is a normal ongoing process in the mammalian brain. It is known that in various neuronal types, including spontaneously firing midbrain dopaminergic neurons, electrophysiological properties can change from young to adult animals. We have investigated this in regard to ion channels involved in pacemaking in the locus coeruleus (LC). Both LC and midbrain dopaminergic neurons have an intrinsic involvement with age-related neurological disorders such as Parkinson's disease. LC degeneration seems to be an early event in this disease that has been reported to occur before damage to dopaminergic neurons. Indeed, these two neuronal types also share common enzymes from the dopamine/noradrenaline synthesis pathway. Due to the key importance of LC neurons in brain function, we compared electrophysiological properties of LC neurons in young and adult mice. The methods used for euthanizing mice were approved by the Animal Care and Ethics Committee at the University of Newcastle. It was found that the resting membrane potential was slightly hyperpolarized in adult animals (n=24), resulting in many of the adult LC neurons were not spontaneously active compared to LC neurons from the young mice (n=25). Input resistance and some of the pacemaking currents also were significantly different in adult compared to neonatal LC neurones. These results suggest that basic electrophysiological properties of LC neurons change with normal development, suggesting this phenomenon is a common process among both dopaminergic and noradrenergic neurons.

## POS-MON-210

### INTERFERON-INDUCED FUNCTIONAL CHANGES IN 5-HT<sub>2C</sub> RECEPTORS AND KV1 CHANNELS IN MOUSE BRAIN

Raymond C.R.<sup>1</sup>, Frese M.<sup>2,3</sup>, Matthaai K.I.<sup>2</sup> and Kole M.H.P.<sup>1</sup>

<sup>1</sup>Neuroscience and. <sup>2</sup>Structural Biology Programs, The John Curtin School of Medical Research, The Australian National University, Canberra. <sup>3</sup>The University of Canberra, Canberra, ACT.

Interferon (IFN) treatment is an effective therapy for a number of diseases, including hepatitis B and C, and multiple sclerosis, but can also produce severe neuropsychiatric side effects. One interesting IFN-induced protein is the p150 isoform of adenosine deaminase acting on RNA (ADAR1). All ADAR proteins are highly expressed in the brain and edit mRNAs of key signalling proteins, including the 5-HT<sub>2C</sub> receptor and Kv1.1 channels. However, a functional role for IFN-induced editing in neuronal physiology has yet to be demonstrated. We have investigated the effect of IFN by injecting mice with the synthetic double-stranded RNA (poly I:C), which results in an immediate and massive IFN $\alpha$  production. In hippocampal pyramidal neurons in brain slices from untreated control mice, activation of 5-HT<sub>2C</sub> receptors with the selective agonist Ro 60-0175 increased the frequency of miniature excitatory postsynaptic currents and the amplitude of the slow after-hyperpolarising potential (n=4, p<0.05). These effects were abolished in slices from poly I:C-treated animals (n=5). Furthermore, poly I:C treatment reduced the fast inactivation of Kv1 currents in axons of cortical pyramidal neurons, resulting in prolonged current availability (n=6, p<0.02). These effects of poly I:C on 5-HT<sub>2C</sub> and Kv1 function were abolished in IFN- $\alpha/\beta$  receptor (IFNAR) knockout mice, confirming a causative role of type 1 IFNs. Our data suggest that functional modulation of 5-HT<sub>2C</sub> and Kv1 could underlie some of the neurological effects of IFN treatment. We are currently investigating the role of ADAR1-mediated editing in this important neural-immune interaction.

## POS-MON-212

### SODIUM AND POTASSIUM CONDUCTANCES IN PRINCIPAL CELLS OF THE PIRIFORM CORTEX

Ikeda K., Suzuki N. and Bekkers J.M.

Neuroscience Program, John Curtin School of Medical Research, The Australian National University.

The piriform cortex (PC), which is only two synapses downstream from the olfactory epithelium, is critical for olfactory information processing. The main input layer of the PC, layer II, contains two main classes of principal neurons: semilunar (SL) cells and superficial pyramidal (SP) cells. These cells exhibit distinctive firing properties, with likely consequences for olfactory information processing. **Aim:** Our aim was to elucidate the ionic mechanisms responsible for the different firing properties of SL and SP neurons. **Methods:** Whole-cell current clamp recordings were made from identified SL and SP cells. Voltage clamp recordings were made from nucleated outside-out patches. **Results:** Sodium current activation and inactivation properties were identical in the two cell types (n=11). TEA (200  $\mu\text{M}$ , n=7) and 4-AP (200  $\mu\text{M}$ , n=9) both eliminated the burst-firing of SP cells, implicating delayed rectifier ( $I_K$ ) and/or A-type ( $I_A$ ) potassium currents.  $I_A$  activated and inactivated at significantly more hyperpolarized potentials in SL cells than in SP cells (activation: SL:  $-36.4\pm1.6$  mV, n=6; SP:  $-25.7\pm1.6$  mV, n=7; p<0.001; inactivation: SL:  $-85.9\pm1.1$  mV, n=7; SP:  $-78.1\pm1.2$  mV, n=5; p<0.001).  $I_K$  activation and inactivation did not differ between SL and SP cells (n=4 each). Finally, apamin increased the firing frequency in both SL and SP cells, but both types expressed a similar amount of apamin-sensitive SK conductance. **Conclusions:** The characteristic firing properties of the two main input neurons of the PC, SL cells and SP cells, are at least partially determined by voltage-gated and calcium-activated potassium channels.  $I_A$ , which differs strongly between SL and SP cells, is likely to be critical for determining the firing phenotype.

## POS-MON-213

**ACTION POTENTIAL BACKPROPAGATION IN CORCAL INTERNEURONS**

Gooch H., **Cavazzini M.G.**, Palmer L., Kole M. and Stuart G., John Curtin School of Medical Research, ANU, Canberra.

Inhibitory interneurons play a critical role in the control of cortical excitation and network synchrony. This is achieved through reciprocal synaptic coupling, as well as dendritic gap junctions and dendritic transmitter release. These latter two processes require robust propagation of action potentials (APs) into the dendritic tree. Here we investigate the efficiency of AP backpropagation in cortical interneurons using voltage-sensitive dyes (VSD). This technique allows the direct recording of transmembrane potential simultaneously at multiple locations, which is difficult to achieve with conventional electrophysiological methods. We focus on cortical layer 2/3 bitufted interneurons, identified by their morphology, firing pattern in response to somatic current injection and somatic AP waveform. After identification, interneurons were filled with VSD (JPW1114) via a somatic recording pipette and fluorescent signals generated in response to APs were recorded at multiple dendritic regions. The amplitude of AP signals at each dendritic location was calibrated by comparing the fluorescent response to hyperpolarizing steady-state voltage changes (generated by somatic current injection) with that predicted from morphologically realistic models. On average, dendritic AP signals attenuated to approximately 50% of the somatic AP amplitude at a distance of 100  $\mu\text{m}$  from the soma ( $n=6$ ). These data demonstrate that APs invade the dendrites of cortical layer 2/3 bitufted interneurons in a decremental manner, suggesting that their impact will be greatest at proximal dendritic locations. Further investigations will examine the role of dendritic voltage-gated channels in regulating AP backpropagation in these neurons.

## POS-MON-214

**THE SLOW-AHP MODULATES BACK PROPAGATION OF ACTION POTENTIALS**

**Power J.M.**, Curby P.G., Bocklisch C. and Sah P. Queensland Brain Institute, University of Queensland, Brisbane, QLD 4072.

In projection neurons of the basolateral amygdala (BLA), trains of action potentials (APs) are followed by a prolonged slow afterhyperpolarization (sAHP) that lasts several seconds and produces pronounced spike frequency adaptation. It is well established that the sAHP results from activation of a slow calcium-activated potassium current ( $\text{sl}_{\text{AHP}}$ ); however, it is controversial as to whether the channels underlying the  $\text{sl}_{\text{AHP}}$  are located on the soma or the dendritic tree. If the channels are located along the dendrite then the sAHP may affect communication between the soma and the dendritic tree. To examine whether the sAHP modulates propagation APs from the soma to the dendrite, whole-cell patch-clamp recordings and high-speed calcium fluorescence images were made from BLA projection neurons in slices obtained from rats (21-28 d). Brief somatic current injections were used to evoke APs that produced a rapid rise in calcium throughout the dendritic tree. When APs were evoked during the sAHP, the AP-induced dendritic calcium response was reduced by  $42 \pm 6\%$  ( $p < 0.01$ ;  $n = 13$ ). APs evoked during a somatic current injections that mimic the hyperpolarisation of the sAHP also reduced the dendritic calcium response to a lesser extent ( $7 \pm 3\%$ ). Stimulation of  $\beta$ -adrenergic receptors reduced the sAHP and the attenuation of the AP evoked calcium rise during the sAHP ( $p < 0.05$ ;  $n = 9$ ). These results show that the backpropagating AP can be modulated by the sAHP. A computational model of a BLA neuron indicates that these results require the presence of dendritic  $\text{sl}_{\text{AHP}}$  channels.

## POS-MON-215

**THE IMPACT OF DENDRITIC SPIKES ON EXTRACELLULAR ELECTROPHYSIOLOGICAL RECORDINGS IN VIVO**

**Bock T.**<sup>1,2</sup>, Murayama M.<sup>1</sup>, Palmer L.M.<sup>1</sup> and Larkum M.E.<sup>1</sup>

<sup>1</sup>Department of Physiology, University of Bern, Switzerland. <sup>2</sup>John Curtin School of Medical Research, ANU Canberra, Australia.

Although the intracellular mechanisms of regenerative electrical activity (such as Calcium and NMDA spikes or back propagating action potentials) in the apical dendrites of layer 5 (L5) pyramidal neurons have been studied intensively during the past decade, the impact of this activity on extracellular electrophysiological recordings in the cortex in vivo is still unknown. Optical recordings of intradendritic Calcium transients have shown that this activity is regulated by inhibition, mediated by deep layer Martinotti cells. In this study we used linear probes and tetrodes in vivo to record evoked extracellular activity before and after local application of tetrodotoxin (TTX,  $n=5$ ) and gabazine ( $n=5$ ) to L5 of the rat somatosensory cortex. Current source density (CSD) and multiunit analysis of these recordings showed that blocking neuronal activity in L5 by applying TTX results in a ~2-fold increase of the current sink in the upper layers, suggesting increased excitatory activity. The application of gabazine decreased the putative excitatory activity (i.e. the sink) in the upper layers. These results are consistent with a previous study, which showed that the application of TTX and gabazine to L5 modulated dendritic Calcium transients in a similar manner. Multi- and single unit analysis of tetrode recordings in the upper cortical layers showed that the firing frequency of layer 2/3 (L2/3) neurons did not change significantly after drug application. Furthermore, in vivo patch clamp recordings of L2/3 neurons showed no change in EPSP duration or amplitude, confirming that excitation in that layer remains constant after application of TTX. We conclude that the change in activity in the upper layers is not dependent on L2/3 cells but rather we suggest that it may be caused by regenerative electrical activity in the apical dendrites of L5 pyramidal neurons.

## POS-MON-216

**IN VIVO TWO PHOTON IMAGING AND ULTRASTRUCTURAL ANALYSIS OF SHORT-TERM DENDRITIC SPINE PLASTICITY IN APICAL DENDRITES OF LAYER V PYRAMIDAL NEURONS**

**Chuckowree J.**<sup>1,2</sup>, Holtmaat A.<sup>4</sup>, Welker E.<sup>2</sup> and Knott G.<sup>2,3</sup>

<sup>1</sup>Menzies Research Institute, University of Tasmania, Australia.

<sup>2</sup>DBCM, University of Lausanne, Switzerland. <sup>3</sup>CIME, EPFL, Switzerland. <sup>4</sup>CMU, University of Geneva, Switzerland.

Recent in vivo imaging studies have shown that a certain fraction of adult dendritic spines appear and disappear on a daily basis. In our previous studies we estimated this fraction to be 20% of the spines in layer I of the barrel cortex (Holtmaat et al. 2005). Additionally, some of these modifications were shown to be associated with an alteration in synaptic connectivity (Knott et al. 2006). Here, we investigated the changes occurring over just a few hours. By placing a cranial window over the barrel cortex of adult mice, we imaged at six hour intervals, the apical dendrites of GFP expressing layer V pyramidal neurons. We counted all laterally extending protrusions occurring on a total of 9253  $\mu\text{m}$  of apical dendrite (11 neurons from 9 mice). After six hours, we found that  $21.4\% \pm 5.2\%$  ( $n=362$ ) of spines had disappeared and  $23.0\% \pm 5.9\%$  ( $n=372$ ) appeared. This is the same fraction of spines that was reported to have been lost and gained over a 24 hour period and suggests that this 'transient' population is more labile than previously thought. The current data suggest that 1 out of 25 spines appears or disappears each hour. To determine how spine formation contributes to alterations in synaptic connectivity, we are currently carrying out ultrastructural analysis using serial section electron microscopy. These data will provide new insights into the mechanisms underlying synaptogenesis and circuit reorganisation in the adult cortex.



## POS-MON-217

**ELECTROPHYSIOLOGICAL PROPERTIES OF HETEROLOGOUSLY-EXPRESSED NAV 1.2 VOLTAGE-GATED SODIUM CURRENTS**French C.<sup>1,2</sup><sup>1</sup>University of Melbourne. <sup>2</sup>Royal Melbourne Hospital.

Voltage gated ion channels composed of human Nav 1.2 alpha subunits were transiently expressed in an HEK cell line and examined under voltage clamp conditions using the whole-cell patch-clamp method. High temporal resolution was achieved performing signal-averaged recordings at up to 100 kHz at reduced temperatures (from 22 to 6°C). Series-resistance artifact was minimized by recording from cells with relatively small amplitude currents (typically <2 nA), low total capacitance (typically <10 pF) and the use of very low resistance pipettes (200-800 kohm), together with both predictive and corrective series-resistance compensation circuitry at high band-width. Activation of the current occurred mono-exponentially without evident delay at potentials up to 20 mV positive to threshold, but developed an inflection with larger depolarisations describing a sigmoidal time-course that was best fitted by an exponential raised to the second power ("m2 kinetics"). Depolarisations activated after variable duration repolarisations during the activation period (~1ms) revealed monoexponential activation at short latency, followed by second order activation with longer pulses. Macroscopic inactivation was comparatively slow and monoexponential at low depolarisations, but developed a bi-exponential time-course with larger depolarisations. The amplitude of the faster time-constant was dominant, approaching a fixed ratio of ~0.8 at maximal activation. A significant persistent component ("INa(p)") was always observed with an amplitude approximately 1% of peak amplitude. Steady-state activation curves were reasonably well fitted with a single Boltzman function with slope and half-activation potential of  $6.4 \pm 0.39$  and  $-27.4 \pm 2.2$  mV (n=6, mean±SEM) respectively. Steady state inactivation studies with conventional 150 ms voltage commands were again reasonably well described by single Boltzman functions with slope and half-inactivation values of  $8.9 \pm 1.4$  and  $-68 \pm 2.2$  mV (n=6). Macroscopic inactivation could be eliminated with intracellular trypsin (0.2 mg/ml). "Slow inactivation" with time constants of the order of 100ms could also be observed, and was preserved with intracellular trypsin. This study provides very high resolution measurements of the kinetic properties of one of the predominant Nav subtypes in the mammalian CNS which are inconsistent with the Hodgkin-Huxley formalism, and demonstrates that complex features of channel behaviour are preserved with solely alpha subunit composition.

## POS-MON-219

**A TWO-STEP PROCEDURE TO FIT SYNAPSE MODELS TO EXPERIMENTAL DATA**Mohan A.<sup>1</sup> and Stricker C.<sup>1,2</sup><sup>1</sup>The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200. <sup>2</sup>ANU Medical School, The Australian National University, Canberra, ACT 0200.

Synaptic modelling is a tool to understand synaptic dynamics and their effect on network behaviour. Parameters of a synapse model can be of three kinds: parameters that can be experimentally determined, those that cannot be measured and those that do not have a directly measurable experimental interpretation. Obtaining accurate model parameters is especially useful in the last two cases because they supplement experimental understanding and help reduce current conceptual limitations. **Purpose:** A phenomenological model for synapse dynamics in rat barrel cortex was utilised (Fuhrmann *et al.*, 2004). In addition to depression caused by vesicle depletion alone, it captures release-independent depression and frequency-dependent recovery and requires 9 free parameters. Fitting this model to experimental data presents major challenges as the parameter space is not uniform, may have discontinuities and several similar solutions may exist. **Results:** We present a straightforward technique to obtain robust solutions. The approach consists of two consecutive steps. 1) To avoid derivatives, Simulated Annealing is used to make an initial parameter guess. It makes almost no model assumptions and converges with high probability to the global minimum. 2) To better constrain the parameter set, global fitting is applied to a family of datasets simultaneously and includes the first two moments of the synaptic response. In simulations, we obtained fits with normally distributed residuals, six orders of magnitude smaller than the response. Unused parameters were correctly identified as zero. **Conclusion:** This two-step procedure could be used to fit models of synaptic dynamics to experimental data robustly. Work is progressing harnessing this approach for experimental data sets and to further constrain the fitting procedure.

## POS-MON-218

**POSTNATAL REFINEMENT OF SYNAPTIC DYNAMICS BETWEEN LAYER 5 PYRAMIDAL NEURONS IN RAT VISUAL CORTEX**Etherington S.J.<sup>1,2</sup> and Williams S.R.<sup>1,3</sup><sup>1</sup>MRC Laboratory of Molecular Biology, Cambridge, UK. <sup>2</sup>Murdoch University, Perth, Australia. <sup>3</sup>Queensland Brain Institute, St Lucia, Australia.

Cortical information flow may be altered by maturational changes in use-dependent synaptic dynamics at intracortical connections. Using multi-neuronal whole-cell voltage recordings, we characterized the development of synaptic dynamics at excitatory connections between layer 5 pyramidal neurons in visual cortex during the first 4 postnatal weeks (n = 158 pairs). In young (P11-15) cortex, unitary EPSPs were large and reliable, with a median amplitude of ~570 uV and a median failure rate of only 9%. In animals P25-29, the median uEPSP amplitude had decreased markedly to ~135 uV, accompanied by an increased coefficient of variation and uEPSP failure rate (34%). Some of these developmental changes in synaptic properties (i.e. increased uEPSP failures) were temporally associated with postnatal eye opening. Mature layer 5 connections showed strong, frequency-dependent paired pulse facilitation across the range of stimulation frequencies tested (10-50 Hz, mean paired pulse ratios between 1.3 and 1.95). In younger cortex, paired pulse depression was observed across the frequency range (mean paired pulse ratios between 0.53 and 0.8) and less variability in paired pulse dynamics was observed. Developmental modification of synaptic dynamics was also manifest during complex action potential trains; P25-29 synapses effectively maintained transmission during prolonged spike trains across a range of frequencies, whereas synaptic potentials in P11-15 cortex depressed rapidly within a few action potentials, except at very low stimulation frequencies (0.2 Hz). Thus, the first postnatal month sees a reduction in the efficacy of transmission of single action potential signals between Layer 5 pyramidal cells in visual cortex, accompanied by improved dynamic range and capacity for transmission of complex spike trains.

## POS-MON-220

**DENDRITIC SPINES PROMOTE SYNAPTIC EGALITARIANISM**Gulledge A.T.<sup>1</sup> and Stuart G.J.<sup>2</sup><sup>1</sup>Dartmouth Medical School, Lebanon, USA. <sup>2</sup>John Curtin School of Medical Research, Canberra, Australia.

Many neurons receive glutamatergic excitatory input almost exclusively onto specialized neuronal processes called dendritic spines. In the absence of spines, the amplitude and kinetics of excitatory postsynaptic potentials (EPSPs) at the site of synaptic input are highly variable and strongly influenced by local dendritic geometry. Here we demonstrate that a fundamental biophysical attribute of spines is to limit location-dependent variability in EPSP properties at the site of synaptic input. In a simplified "ball and stick" model EPSPs onto spines showed limited variability in amplitude, peak latency, and half-width within the spine head, while the same synaptic inputs made directly onto the dendritic shaft generated EPSPs with highly variable amplitude and shape. The coefficient of variation (CV) of local EPSP amplitude, peak latency, and half-width were 0.087, 0.090, and 0.092, respectively, for input onto spines (neck resistance=200 MΩ) compared to 0.820, 0.302, and 0.332, respectively, for identical EPSPs onto dendritic shafts at the same locations. The impact of spines on local EPSPs was largely independent of synaptic conductance, but negatively correlated with spine neck resistance. Synaptic input onto spines with high neck resistance showed less EPSP variability compared to inputs onto spines with lower neck resistance. Similar observations were made in morphologically realistic models. We propose that one function of spines is to standardize the amplitude and kinetics of local EPSPs, making them less dependent on synapse location within the dendritic tree. Because EPSPs can activate voltage-dependent channels, such as NMDA receptors, the ability of spines to standardize the local EPSP voltage independent of synapse location will allow neurons to utilize similar postsynaptic mechanisms at all synaptic locations.

## POS-MON-221

## INTERACTIONS BETWEEN CORTICAL INHIBITION AND SHORT-INTERVAL CORTICAL FACILITATION (SICF)

Cash R.F.H.<sup>1</sup>, Ziemann U.<sup>2</sup> and Thickbroom G.W.<sup>1</sup><sup>1</sup>Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Australia. <sup>2</sup>Department of Neurology, Goethe-University of Frankfurt, Germany.

Transcranial magnetic stimulation (TMS) over motor cortex can elicit multiple excitatory and inhibitory effects including the trans-synaptic activation of principle cells at high frequency (indirect (I)-waves; ~1.5ms periodicity), and short- and long-interval cortical inhibition (SICI, LICI) thought to involve GABA<sub>A</sub> and GABA<sub>B</sub> receptors that may be located pre- or post-synaptically. These effects can interact in multiple ways, e.g. short interval cortical facilitation (SICF) between I-waves with paired pulses delivered at I-wave intervals. In the present study we explored the interaction of inhibitory circuits with the excitatory circuits that control SICF. Seven healthy subjects were recruited (20-38 years of age). We used a triple-pulse TMS protocol to investigate the effect on SICF (paired-pulse; 1.5ms inter-pulse interval (IPI)) of a suprathreshold priming stimulus (PS) sufficient to induce LICI, at PS-SICF intervals of 100-300ms. Adjustments were made to account for the direct excitability effects induced by PS. PS initially had no effect on SICF, however this was followed by a late phase from 190-220ms, coinciding with the end of LICI, when SICF was increased (up to  $189 \pm 29\%$ ,  $p < 0.01$ ). We conclude that there is a late post-inhibitory phase during which the networks associated with trans-synaptic activation of excitatory interneurons are facilitated, possibly as a result of cortical disinhibition.

## POS-MON-222

## TWO LAYERS OF SYNAPTIC PROCESSING BY PRINCIPAL NEURONS IN THE PIRIFORM CORTEX

Suzuki N. and Bekkers J.M.

Neuroscience Program, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia.

The piriform cortex (PC) is an anatomically-simple three-layered cortex that processes olfactory information. The main input layer of the PC, layer II, contains roughly equal numbers of two classes of glutamatergic principal neurons: semilunar (SL) cells and superficial pyramidal (SP) cells. Both classes are known to receive afferent (*aff*) input from the olfactory bulb and associational (*assn*) input from other PC principal neurons. **Purpose:** Our aim was to compare *aff* and *assn* synaptic inputs onto SL and SP cells to assess the involvement of each cell type in processing afferent versus intracortical information. **Methods:** Experiments used 300  $\mu$ m-thick slices of PC from 14-25 d-old mice. Dual whole-cell recordings from identified SL and SP cells were accomplished using standard methods. **Results:** Bulk extracellular stimulation of *aff* inputs elicited excitatory postsynaptic currents (EPSCs) that were  $1.6 \pm 0.2$  ( $n=5$  pairs) times larger in SL cells, whereas stimulation of *assn* inputs elicited EPSCs that were  $7.7 \pm 1.8$  ( $n=6$  pairs) times larger in SP cells. Minimal extracellular stimulation showed that unitary EPSCs were significantly larger in SL cells with *aff* stimulation (SL:  $70.7 \pm 9.5$  pA,  $n=29$ ; SP:  $19.4 \pm 2.3$  pA,  $n=20$ ;  $p < 0.01$ ) but of similar size with *assn* stimulation (SL:  $42.6 \pm 4.9$  pA,  $n=10$ ; SP:  $41.3 \pm 8.4$  pA,  $n=10$ ;  $p=0.89$ ). Finally, polysynaptic *assn* inputs, provoked by disinhibition, were prominent in SP cells but not in SL cells. **Conclusions:** SL cells receive stronger input from the olfactory bulb, whereas SP cells receive stronger intracortical input. Hence, the PC contains two functionally distinctive input layers: one comprising SL cells, the other comprising SP cells.

## POS-MON-223

## OPIOID AND CANNABINOID DISINHIBITION OF A DESCENDING ANALGESIC PATHWAY IN THE PERIAQUEDUCTAL GREY

Lau B.K. and Vaughan C.W.

Pain Management Research Institute (PMRI), Kolling Institute of Medical Research, Northern Clinical School (University of Sydney).

**Purpose:** The Periaqueductal Grey (PAG) is a major site of analgesic action of opioids and cannabinoids. These agents have long been hypothesized to predominantly produce analgesia via an indirect process of disinhibition in descending analgesic systems. Of particular interest is a descending pathway that projects through the PAG via the Rostral Ventromedial Medulla (RVM) to modulate nociceptive transmission at the Spinal Cord Dorsal Horn. Many studies suggest that  $\mu$ -opioids produce disinhibition in the PAG-RVM descending pathway, however there is no direct evidence demonstrating disinhibition of PAG output neurons projecting to the RVM. Thus, there still remains a lack of definitive support for disinhibition of the principal neurons involved in antinociception. The present study aims to address this issue by examining the cellular actions of analgesic agents like opioids and cannabinoids on PAG-RVM output neurons. **Methods:** PAG output neurons projecting to the RVM were retrogradely labelled. Electrophysiological whole-cell patch clamp recordings were then conducted from these identified PAG output neurons. Paired recordings between inhibitory (and excitatory) interneurons and principal output neurons were also performed. **Results:** The  $\mu$ - and  $\kappa$ -opioid agonists, DAMGO and U69593 produced a reduction of evoked inhibitory postsynaptic currents (IPSCs) in PAG output neurons, while the  $\delta$ -opioid agonist, deltorphan had no significant effect. Hence, both  $\mu$ - and  $\kappa$ -opioid agonists act presynaptically to suppress inhibitory GABAergic synaptic transmission onto PAG output neurons. **Conclusions:** We have previously shown that only a small proportion of output neurons in the ventrolateral PAG respond directly to opioid agonists. This finding in combination with the present observations is consistent with the opioid disinhibition model of descending analgesia.

## POS-MON-224

## QUALITATIVE COMPARISON OF PHASE RESPONSE CURVE ESTIMATION METHODS USING MODEL AND EXPERIMENTAL DATA

Torben-Nielsen B., Uusisaari M. and Stiefel K.M.

Theoretical and Experimental Neurobiology Unit, Okinawa Institute of Science and Technology, 12-22, Suzaki, Uruma, Okinawa 904-2234, Japan.

The phase-response curve (PRC) associated with a neuron is generally believed to reflect the type of excitability of this neuron. A neuron having a purely positive PRC (type I PRC) would exhibit type I excitability while a PRC containing negative region(s) (type II PRC) would be indicative of type II excitability. Furthermore, the type of excitability of neurons in a network can predict the extent of synchronization within the network. Several methods are in use to determine the PRC from either experimental data or modeled data, but their relative efficiency and accuracy has not been examined. We implemented and compared four methods and assessed the limitations and opportunities of the different methods especially in the context of usefulness in analyzing actual experimental data. We found that (i) all methods require non-standard physiological data and hence a specialized stimulation protocol, and (ii) considerable variance in the PRCs originates from the initiation of parameters as well as the use of different methods. Moreover, we performed a sensitivity analysis of the PRC with respect to several intrinsic neuronal properties. This study contributes to a better understanding of the nature of neuronal excitability and its relation to PRC curves.

## POS-MON-225

## WHITE NOISE CONDITIONING OF THE VESTIBULAR EVOKED MYOGENIC POTENTIAL (VEMP)

Chong H. and Burne J.

Discipline of Biomedical Science, School of Medical Sciences, Faculty of Medicine, The University of Sydney, PO Box 170, Lidcombe, NSW, 2141, Australia.

**Background:** The vestibular evoked myogenic potential (VEMP) is an acoustically evoked potential recorded from the sternocleidomastoid muscle (SCM). It is used clinically to test the integrity of the otolith organs and inferior vestibular nerve based on lesioning evidence that the response is mediated by the otoliths, especially the saccule. Although there is a literature on the effects of prior loud noise exposure on hearing thresholds (masking effects), there has been little investigation of the effects of loud noise exposure on the VEMP and no such mechanism for this has been postulated. **Aim and method:** This study investigated the conditioning effects of a brief exposure to loud white noise on the VEMP amplitude. A virtual instrument was developed on a Labview platform to generate a 25ms white noise conditioning stimulus and a pure tone test stimulus (500Hz, 2ms). These stimuli were presented in paired format over a range of interstimulus intervals (<225ms) to the ear ipsilateral to the recorded and averaged ( $n > 100$ ) SCM evoked VEMP. **Results:** In six tested subjects, white noise conditioning significantly ( $p < 0.05$ ) reduced the peak to peak amplitude of the VEMP n13p23 response (>50%) at all 5 tested ISIs to 225ms. Maximum inhibition occurred at an ISI of 25ms. **Conclusion:** Prior white noise conditioning produced a pronounced and prolonged depression of the VEMP amplitude. This result prompts further consideration as to the possible sites for interaction between the presumed vestibular test stimulus and auditory conditioning stimulus.

## POS-MON-227

## A PREPARATION FOR STUDYING AXON REGENERATION AND DESCENDING SYNAPTIC CONNECTIONS AFTER SPINAL CORD INJURY IN MICE

Flynn J.R.<sup>1</sup>, Galea M.J.<sup>2</sup>, Brichta A.M.<sup>1</sup>, Callister R.J.<sup>1</sup> and Graham B.A.<sup>1</sup>

<sup>1</sup>The University of Newcastle, Callaghan, NSW. <sup>2</sup>The University of Melbourne, Melbourne, VIC.

Recent evidence suggests manipulation of molecular pathways and exercise can improve function after spinal cord injury (SCI), presumably by plasticity associated with axon regeneration and sprouting (Goldshmit et al., 2004 J Neuroscience 24: 10064). Surprisingly, we know little about the synaptic connections regenerating or sprouting axons make to "bridge" a spinal cord lesion. **Purpose:** To develop a horizontal spinal cord slice preparation for electrophysiological examination of synaptic connections between descending axons and spinal neurons. **Methods:** Mice (C57Bl/6, > P19-41) were anaesthetised (Ketamine 100 mg/kg i.p.) and decapitated. Horizontal slices (300  $\mu$ m thick) containing T8-L4 spinal segments were cut and whole-cell recordings were obtained from visualized neurons in the intermediate zone ( $KCH_3SO_4$  internal, at 23°C). Evoked synaptic responses were obtained by stimulating the dorsal columns at various distances rostral to the recording site. In mice, the dorsal columns contain corticospinal and propriospinal axons. **Results:** Synaptic responses were evoked in 26 of 32 recordings. The separation between stimulating and recording sites ranged from 0.3-1.9 mm. In voltage-clamp, three different types of responses were observed: single component monosynaptic (15/26); dual component monosynaptic (8/26); and multi component polysynaptic (3/26). Subsequent current-clamp recordings showed some responses contained an inhibitory component (5/13). A range of action potential discharge patterns was also observed in neurons that demonstrated evoked synaptic responses: Tonic firing (5/13); Initial bursting (6/13); and Delayed firing (2/13). **Conclusions:** The *in vitro* horizontal slice preparation could be used for future study of descending synaptic connections to spinal neurons in both normal mice and in those demonstrating functional recovery after SCI.

## POS-MON-226

## USING PHOTORHODOPSINS TO PROBE NEURONAL CIRCUITRY

Gooch H.M., Sedlak P. and Sah P.

The Queensland Brain Institute, The University of Queensland, St Lucia.

**Introduction:** The advent of photorhodopsin neuroengineering heralds a new epoch in the investigation of neuronal-circuit functionality and interconnectivity. Derived from microbial opsins, these proteins are light-activated transmembrane structures with the capacity to confer bidirectional modulatory control over neuronal activity. These tools are temporally precise, produce no electrical artefact and, therefore, represent a powerful method for the investigation of synaptic plasticity. **Purpose:** We are working to establish the use of photorhodopsins to probe the neuronal circuitry of the amygdala, a region of interconnected nuclei that is crucial for both the acquisition and storage of emotional memory. **Methods:** Fluorophore-fused cDNA constructs of Channelrhodopsin-2 (hChR2), Volvox (VChR1) and Halorhodopsin (NpHR) were transfected into both HEK293T and rat hippocampal primary culture. Using whole-cell patch clamp techniques, hChR2- and VChR1-expressing HEK293T cells were illuminated with photorhodopsin-specific excitation wavelengths and the resulting photocurrent amplitudes recorded. Third generation lentiviruses were produced for each construct and thalamic injections undertaken in 18-20 day old wistar rats. Following a 4-6 week recovery period, photorhodopsin expression was further characterised. **Results:** The transfection of both HEK293T and hippocampal cultures confirmed membrane-bound fluorescence for each of the photorhodopsin constructs. Whole-cell photocurrents of up to 1 nA were recorded in HEK293T cells ( $n = 2$ ) transfected with VChR1. Lentivirus titres were calculated in the range of  $8 \times 10^7$  infectious units/ml. Animals stereotactically injected with hChR2 lentivirus into the thalamus (MGN) showed neuronal expression 5 weeks after injection, detectable via fused-fluorophore imaging. **Conclusion:** This data supports the use of light-activated proteins within the amygdala to provide new insight into the acquisition and storage of emotional memory.

## POS-MON-228

## RECEPTOR-MEDIATED GENE DELIVERY INTO MICROGLIA VIA SCAVENGER RECEPTOR CLASS B, TYPE I

Malmevik J.M.<sup>1</sup>, Rogers M.-L.<sup>1</sup>, Nilsson M.<sup>2</sup>, Nakanishi Y.<sup>3</sup>, Rush R.A.<sup>1</sup>, Sims N.R.<sup>1</sup> and Muyderman H.<sup>1</sup>

<sup>1</sup>Centre for Neuroscience, Flinders Medical Science and Technology, Flinders University, Australia. <sup>2</sup>Institute for Neuroscience, Goteborg University, Sweden. <sup>3</sup>Faculty of Pharmaceutical Sciences, Kanazawa University, Japan.

Microglia constitute the major inflammatory cell type of the central nervous system (CNS). Progress in understanding the microglial response in the diseased CNS is restricted by limited approaches allowing this cell type to be selectively targeted within the complex environment of the mature brain. Viral vectors have been used to modify microglial function; however the outcomes of such studies have been disappointing. Non-viral vectors offer an alternative approach in better targeting some cell populations and avoiding immune responses that can be produced even by highly modified viral vectors. Receptor-mediated gene delivery constitutes one such approach. In this study, we evaluated the potential of selectively targeting microglia *in vivo* utilising receptor-mediated gene delivery via the scavenger receptor class B type I (SR-BI). A majority of microglial cells were demonstrated to express the SR-BI receptor both *in vitro* and *in vivo*. Moreover, microglial cells *in vitro* rapidly internalised an antibody targeted at the extra-cellular domain of SR-BI. Intracerebral injections of the antibody resulted in selective microglial uptake. The SR-BI antibody was then linked to the polycation polyethylenimine and bound to a CMV promoter-driven plasmid encoding for green fluorescent protein (GFP). Exposure with this *immunogene* resulted in GFP expression in a few microglial cells *in vitro*. In contrast, intrahippocampal infusions of the *immunogene* ( $n = 3$ ) resulted in a substantial microglial GFP expression, demonstrating for the first time the use of a non-viral transfection system to selectively target the microglial cell population *in vivo*.



## POS-MON-229

# TRANSFECTION EFFICIENCY AND TOXICITY OF P75<sup>NTR</sup> TARGETED NON-VIRAL GENE DELIVERY IN WILD TYPE AND SOD1<sup>G93A</sup> PRIMARY MOTOR NEURON CULTURES

Matusica D.<sup>1</sup>, Rogers M.-L.<sup>1</sup>, Muyderman H.<sup>2</sup> and Rush R.A.<sup>1</sup>

<sup>1</sup>Department of Human Physiology, Centre for Neuroscience, School of Medicine, Flinders University, GPO Box 2100 Adelaide 5001. <sup>2</sup>Department of Medical Biochemistry, Centre for Neuroscience, School of Medicine, Flinders University, GPO Box 2100 Adelaide 5001.

**Purpose:** Non-viral gene delivery vehicles offer the possibility of safer therapeutic agent development for neurological conditions such as motor neuron disease (MND). Here we characterize the culture conditions, transfection efficiency and toxicity of non-viral gene delivery constructs selectively targeting p75<sup>NTR</sup> expressing motor neurons using primary embryonic motor neuron (PMN) cultures and mixed cultures (PMNm) from wild type and SOD1<sup>G93A</sup> transgenic mice. **Methods:** Monoclonal antibody to the neurotrophin receptor p75<sup>NTR</sup> (MLR2) was conjugated to polyethylenimine (MLR2-PEI) or pegylated polyethylenimine (MLR2-PEG-PEI) and complexed with an eGFP expression plasmid at nitrogen/phosphate (NP) ratios ranging from 2-10 to form the immunoconjugate, MLR2-PEI-pGFP/MLR2-PEG-PEI-pGFP. Transfection efficiency, toxicity and stability of various NP ratio conjugates were assessed at 72 hours in wild type and SOD1<sup>G93A</sup> PMN and PMNm cultures at day 5-10 after plating and transfected for 4, 24 and 48 hours. **Results:** MLR2-PEI-pGFP and MLR2-PEG-PEI-pGFP are most stable and less toxic at NP ratios of 3.5 and 7 ( $n=6$ ). Both immunogene constructs specifically transfect PMN and PMNm at a transfection efficiency of 2% and 5% respectively ( $n=3$ ), however only MLR2-PEG-PEI-pGFP transfected cultures in the presence of 10% serum containing media ( $n=4$ ). In PMNm cultures containing primarily astrocytes, only motor neurons were transfected ( $n=3$ ). In addition MLR2-PEG-PEI-pGFP NP ratio 3.5-7 constructs were not toxic to PMN cultures as observed with MLR2-PEI-pGFP ( $n=4$ ), however all immunogene constructs were 100% toxic to PMN cultures up to 4 days after plating. **Conclusion:** Pegylation of the MLR2-PEI-pGFP construct produces a gene delivery vehicle with reduced toxicity, improved stability and transfection efficiencies in wild type and SOD1<sup>G93A</sup> PMN cultures. Reduced toxicity and stability of the immunopore may be important in developing gene therapies that target injured or dying motor neurons in MND patients.

## POS-MON-230

# A METHOD FOR MEASUREMENT OF DYNAMIC MIDDLE CEREBRAL ARTERY PRESSURE IN A RAT STROKE MODEL

McLeod D., Calford, M. and Spratt N.  
University of Newcastle.

There are no current ischaemic stroke models in rodents that have the capability of measuring intracerebral haemodynamics on a beat-by-beat basis. The present study describes a microcatheter-based model for measurement of middle cerebral artery (MCA) pressure in rats combined with blockade of blood flow through the MCA. The catheter device consists of a heat-blunted 5-0 monofilament thread (tip diameter 330 micrometers) that is introduced into a polyimide microtube (outer diameter 350 micrometers, length 30mm). The microtube with inserted thread is then secured to a 10 cm length of 1F silicone tube with epoxide glue. The catheter device is then filled with heparinised saline and connected to a fluid filled pressure transducer and data acquisition system. To induce MCA occlusion (MCAo), the catheter is introduced via the external carotid artery and internal carotid artery to simultaneously block blood flow to the anterior cerebral artery (ACA) and MCA. The lumen of the catheter sits at the bifurcation of the ACA and MCA for measurement of MCA pressure. In the present pilot study MCA pressure, femoral arterial pressure and HR were simultaneously measured for 2 hours following micro-catheter MCAo in spontaneously hypertensive (SH) ( $n=1$ ) and outbred Wistar rats ( $n=1$ ) under isoflurane anaesthesia. In both rats, systolic femoral arterial pressure was identical to systolic blood pressure (BP) measured by the microcatheter when initially inserted into the common carotid artery, prior to MCAo. In the SH rat, the micro-catheter systolic BP fell from 145 mmHg to 39 mmHg on MCAo, and from 104 mmHg to 39mmHg in the Wistar rat. This is the first study to measure dynamic changes in MCA pressure in a rodent stroke model.

## POS-MON-231

# EFFECTS OF ATTENTION ON MULTIFOCA PUPILLOGRAPHIC RESPONSES

Rosli Y.<sup>1,2</sup>, Maddess T.<sup>1</sup>, Ho Y.<sup>1</sup>, Wong S.<sup>1</sup>, Kolic M.<sup>1</sup>, Goh X.L.<sup>1</sup>, Carle C.<sup>1</sup> and James A.C.<sup>1</sup>

<sup>1</sup>Centre for Visual Sciences and ARC Centre of Excellence in Vision Science, Research School of Biology, Australian National University, Canberra, Australia. <sup>2</sup>Dept. of Biomedical Science, Faculty of Allied Health Sciences, UKM Jln Raja Muda Abdul Aziz, Kuala Lumpur Malaysia.

**Purpose:** Multifocal pupillometry has recently been developed and refined for visual field assessment in glaucoma. This study explored the presence of attention-related changes to multifocal pupillary activity. **Methods:** Two experiments were carried out, the manipulated variable was the colour of the stimulus (white/yellow); first experiment ( $n=18$ ) at 288cd/m<sup>2</sup> luminance, second experiment ( $n=22$ ) at 150cd/m<sup>2</sup> luminance. Subjects had to fixate a cross at the centre of stimulus, and on some trials responded by clicking a button whenever the cross changed into a dot, while their pupil diameters are monitored. Test stimuli from both protocols were presented at each region at 33 ms per flash; 44/s/eye. Each protocol was divided into eight 30s segments. **Results:** Attention reduced pupil constriction responses when using white stimuli ( $-1.58\mu\text{m}$ ,  $p=0.0001$ ) but increased constriction for yellow stimuli ( $1.15\mu\text{m}$ ,  $p=0.006$ ). These results were verified in Experiment 2: white stimulus responses were suppressed prominently the inner two eccentricity rings: Ring1:  $-0.25\text{dB}$ ; Ring2:  $-0.38\text{dB}$ ; and Ring5 ( $-0.28\text{dB}$ ) relative to responses with yellow stimuli. The foremost difference of Experiment 1 was that white attentional responses were consistently suppressed at all quadrants and eccentricities. **Conclusion:** Pupillary responses were found to be significantly influenced by attention albeit differently for white and yellow stimuli. The overall results seem to suggest that attentional responses were either enhanced or suppressed across quadrants and concentric rings though the degree of suppression and quadrants may differ.

## POS-MON-232

# AUTOMATED ANALYSIS OF MULTIDIMENSIONAL BRAIN IMAGES

Tuxworth G.<sup>1,2</sup>, Alavi A.<sup>1,2</sup>, Nguyen M.<sup>1</sup>, Cavanagh B.<sup>1</sup>, Blumenstein M.<sup>2</sup>, Mackay-Sim A.<sup>1</sup> and Meedeniya A.C.B.<sup>1</sup>

<sup>1</sup>National Centre for Adult Stem Cell Research, ESKITIS Institute, Griffith University, Nathan, Australia. <sup>2</sup>ICT, Griffith University, Gold Coast, Australia.

Neural cells are highly plastic, mirroring their functional state in their morphology. Data from the classification of three dimensional images from individual cells enable the functional state of the cell to be inferred. Due to the complexity of the data, namely, the irregularity in neuronal shape, existing three dimensional segmentation and feature extraction techniques do not perform well. A method was developed for stereological quantification of multiple neural cell classes, obtained from high resolution 3D images. Further data was extracted from these dopaminergic cell types within the substantia nigra ( $n=25$ ), ventral tegmental area ( $n=50$ ), and the olfactory bulb ( $n=25$ ) of the rodent brain ( $n=15$ ) on multiple features of the cell soma. The data on these features were fed into a neural network which was trained to identify each cell type based on its morphology. On completion of training, the neural network was able to distinguish three cell types to within 91% accuracy. It outperformed a human expert in accuracy (73%) and speed, on the same set of data ( $p<0.025$ , t-test). A custom image analysis tool was also developed for investigating nerve fiber density within specific brain and spinal cord regions as well as major peripheral nerve trunks. This allowed the rapid unbiased estimation of absolute nerve innervation density in specific disease models/therapies. The ever increasing dimensionality and quantity of image data demands the image analysis process to be automated. We have demonstrated that the application of automated feature extraction software together with neural network algorithms has facilitated the rapid and unbiased analysis of neural tissue.

## POS-MON-233

**PROGRESSIVE STRUCTURAL AND FUNCTIONAL CEREBRAL CHANGES FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT**

Cardamone L.<sup>1</sup>, Liu Y.R.<sup>1</sup>, Hogan R.E.<sup>2</sup>, Gregoire M.C.<sup>3</sup>, Williams J.P.<sup>4</sup>, Hicks R.J.<sup>5</sup>, Jones N.C.<sup>1</sup>, Myers D.E.<sup>1</sup>, Boullieret V.<sup>6</sup> and O'Brien T.J.<sup>7</sup>

<sup>1</sup>Department of Medicine (RMH), University of Melbourne, Australia  
<sup>2</sup>Department of Neurology, Washington University, USA  
<sup>3</sup>Australian Nuclear Science and Technology Organisation, Menai, Australia  
<sup>4</sup>Small Animal MRI Facility, Florey Neurosciences Institute, Victoria, Australia  
<sup>5</sup>The Centre for Molecular Imaging, Peter MacCallum Cancer Centre, Victoria, Australia  
<sup>6</sup>Department of Neurophysiology and Epilepsy, APHP, CHU Bicêtre, Paris, France  
<sup>7</sup>Department of Neurology, Royal Melbourne Hospital, Australia.

Traumatic brain injury (TBI) has a high incidence of long-term morbidity, with the hippocampus believed to play a key role. This study investigated longitudinal structural and metabolic changes in the rat brain following TBI using MRI and PET. Rats underwent a 3.5atm lateral fluid percussion (FPI; n=16) or sham injury (n=11). MRI and PET were performed at baseline, 1 week, 1, 3 and 6 months post-FPI. Morphological changes were assessed using region-of-interest (ROI) analysis and hippocampal surface changes assessed using large-deformation high-dimensional-mapping (HDM-LD). Metabolic changes were assessed using manual co-registration of ROIs with PET and Statistical Parametric Mapping (SPM). Volumetric changes were observed in the ventricles ( $p=0.03$ ), cortex ( $p=0.0001$ ) and hippocampus ( $p=0.0001$ ) ipsilateral to injury. HDM-LD showed a global hippocampal decrease predominantly ipsilateral to injury, with differential evolution of hippocampal surface changes between hemispheres. PET showed hypometabolism in FPI rats in the ipsilateral cortex and hippocampus which evolved up to 6 months. SPM showed metabolic changes further involved the striatum at one week ( $p=0.007$ ) and part of the contralateral posterior lateral cortex at one month ( $p=0.002$ ). These results demonstrate dynamic and evolving changes post-FPI with widespread focal hypometabolism in specific regions, some remote from the direct trauma site and not detected on MRI. These findings may have implications for understanding the long-term consequences of TBI.

## POS-MON-235

**A NOVEL INTRALUMINAL FIBRE OPTIC CATHETER RECORDS HIGH RESOLUTION LONGITUDINAL AND CIRCUMFERENTIAL GASTROINTESTINAL MOTILITY IN ISOLATED MAMMALIAN INTESTINE**

Dinning P.G.<sup>1</sup>, Arkwright J.<sup>2</sup>, Szczesniak M.M.<sup>1</sup>, Brookes S.J.<sup>3</sup>, Costa M.<sup>3</sup> and Spencer N.J.<sup>3</sup>

<sup>1</sup>Department of Gastroenterology, The St George Hospital, University of New South Wales. <sup>2</sup>Material Science and Engineering, CSIRO, NSW. <sup>3</sup>Department of Human Physiology, School of Medicine, Flinders University, South Australia.

Motor patterns in isolated segments of intestine have been typically recorded from single intraluminal pressure recordings or sparse force transducers attached the outside of the gut wall. These sparse recordings do not enable detection of the full dynamic range of intestinal motor events. To overcome this, we have developed novel intraluminal fibre optic catheters (3mm OD) designed to detect both longitudinal and circumferential contractions at multiple regular closely spaced (15mm) sites. Segments of guinea-pig proximal colon (of 8 cm; n = 5) and of rabbit small intestine (30 cm; n=3) were placed in an organ bath with Krebs at 36°C. Motor activity recorded by the optical catheters was compared with video spatio-temporal mapping of wall motion recorded in parallel. In the guinea pig colon propagated events (velocity 5.4mm/s), and synchronous events were detected by both optical manometry and spatio-temporal mapping. In rabbit small intestine erythromycin (10-6M) elicited slowly propagated contractions revealed by both optical manometry and spatio-temporal maps. In addition both optical catheter and spatio-temporal mapping distinguished well periods of pendular movements due to longitudinal muscle contractions from periods with associated circular muscle contractions. Conclusions: Fibre optic catheters can be used to monitor changes in both longitudinal and circular contraction from multiple sites within isolated segments of intestine. This technology, in combination with video recording, opens a new window for investigating complex gastrointestinal motor patterns in isolated segments of mammalian intestine integrating kinetic and kinematic motor events.

## POS-MON-234

**MAGNETIC RESONANCE SPECTROSCOPIC VARIATIONS BETWEEN HEMISPHERES**

Bull N.<sup>2,1</sup>, Stanwell P.<sup>3,2</sup> and Hunter M.<sup>1,2</sup>

<sup>1</sup>School of Psychology, University of Newcastle, NSW. <sup>2</sup>Hunter Medical Research Institute, NSW. <sup>3</sup>Brigham and Women's Hospital, Boston, MA, USA.

Background: There has been a marked and rapid increase over the last decade in the use of functional magnetic resonance imaging (fMRI) techniques to image brain activation patterns in vivo. The technique has enabled the measurement and localisation of activation patterns in brain states when the participant is engaged in thought processes and responses to stimuli. The BOLD (blood oxygen level dependent) response of fMRI is an indirect measure of neural tissue activation but assumes that metabolic turnover varies according to activation of the tissue. The question then arises can the MR signal be used to further identify these metabolic changes? In the current experiment we use MR spectroscopy (MRS) to investigate this suggestion. Method: Ten normal healthy volunteer participants with equal numbers of left and right-handers were recruited and underwent MRS in a Siemens' 1.5T whole body scanner at the John Hunter Hospital, Newcastle. Voxels (1cm<sup>3</sup>) were centred on the motor cortex hand area of the frontal cortex. Measurements were taken serially during periods of rest and finger tapping with either left or right hand during measurements of left and right hemisphere cortices. Water balance measures were also taken at each measurement. Results: Some variation in the spectra was found between active and inactive (rest) patterns. Of perhaps greater interest, however, was the finding of differences dependent on which hemisphere was the dominant hemisphere. These differences suggest that variations in macromolecules are dependent on hemispheric dominance.

## POS-MON-236

**ILLUMINATING PHD RESEARCH AS A CAREER PATH FOR UNDERGRADUATES**

McAllan B.M., Mackertich M., Pearlman A., Simmons E., Treble A. and Phillips W.D.

School of Medical Sciences (Physiology), University of Sydney 2006.

Over the past decade major structural changes have washed over physiology departments at Australian universities. Changes include large increases in undergraduate student enrollments and the transition to graduate entry into medical training. For Sydney University (at least), these changes have been accompanied by fewer students progressing from the Honours year into PhD studies in Physiology (AuPS News, March 2009). The Biovideo project is an attempt to improve our understanding of undergraduate perceptions of the life of a practicing scientist, and of how their perceptions might influence their decision to embark on a research degree. In 2009, four third-year Neuroscience undergraduates undertook the project with guidance and support from two Physiology academics (BM and WP). Eight undergraduates took part in focus group qualitative research sessions. These discussions suggested that many undergraduate students have no idea what a career in science entails (while medicine apparently offered a much clearer career path). Students felt that an impediment to such knowledge was the lack of small-group and individual contact with academic-researchers due to large class sizes. In the second part of the project the students recruited and interviewed people at various stages of a career in biomedical sciences. They devised a set of questions to probe the personal experience and motivations that led each individual to pursue research. The interviews were video taped and edited to 2 minutes for YouTube. Our intention is that these interviews might form the starting point for a supra-institutional library of video micro-biographies to help future physiology undergraduates gain a clearer idea of what a life in research can offer. The initial biovideos can be accessed via <http://www.physiol.usyd.edu.au/~billp/>.

## POS-MON-237

## USING A PANEL OF IMMUNO AND HISTOCHEMICAL MARKERS TO MAP THE MARMOSET AMYGDALA

Watson C.<sup>1</sup>, Paxinos G.<sup>1,2</sup>, Tokuno H.<sup>3</sup> and Hobbs M.<sup>4</sup><sup>1</sup>Prince of Wales Medical Research Institute. <sup>2</sup>University of New South Wales. <sup>3</sup>Tokyo Metropolitan Institute for Neuroscience. <sup>4</sup>University of Western Australia.

We have used the staining patterns revealed by markers in the rat amygdala (Paxinos et al 2009) to define homologous nuclei in the amygdala of the marmoset (*Callithrix jacchus*). We have examined serial sections stained with the following markers in rotation - SMI32 (SMI), tyrosine hydroxylase (TH), NADPH diaphorase (NADPH-d), parvalbumin (Pv), calbindin (Cb), calretinin (Cr), and acetylcholinesterase (AChE), in addition to Nissl (Ni) staining. We have worked on the assumption that the pattern of protein markers is conserved in mammalian evolution, so that the rat marker series can be used as a kind of Rosetta stone for other species. The amygdala in the marmoset is at first sight very different to that of the rat, because of the rotation of the temporal lobe. However, the markers quickly reveal the probable homologues of the main amygdaloid nuclei. AChE and Pv staining of the three major parts of basolateral nucleus (BL) in the marmoset (BLD, BLI, and BLV) show clearly that they are homologous with the three named parts of BL in the rat (BLA, BLP, and BLV). Dense NADPH-d staining identifies the medial amygdaloid nucleus, the amygdalohippocampal area, and the basomedial amygdaloid nucleus, anterior part (BMA; called BMNC in primates) in both species. In the central nucleus, the lateral part (CeL) is AChE negative in both species. These findings, combined with supplementary data from the remaining markers, make it possible to identify all of the major amygdaloid nuclei in the marmoset. This technique is particularly valuable in situations where few data on connections or electrophysiology are available.

## POS-MON-238

## IDENTIFICATION OF SODIUM-HYDROGEN REGULATORY FACTORS IN THE CHOROID PLEXUS

Rayfield A.<sup>1</sup>, Poronnik P.<sup>2</sup>, Pow D.V.<sup>3</sup> and Lee A.<sup>3</sup><sup>1</sup>SBMS, The University of Queensland. <sup>2</sup>Pharmaceutical Sciences, RMIT. <sup>3</sup>UQCCR, The University of Queensland.

The choroid plexus is a vascular structure arising from the walls of the ventricles of the brain and is responsible for cerebrospinal fluid (CSF) formation. Each choroid plexus consists of a mass of capillaries, invested by modified ependymal cells. A key aspect of CSF secretion involves active secretion of sodium ions, which drives a passive water flux. By analogy, key scaffold proteins involved in regulating sodium flux in tissues such as kidney are the sodium-hydrogen exchange regulatory factors NHERF1 and NHERF2. We have examined their distribution in the choroid plexus. We demonstrate that NHERF2 is associated with the endothelial cells whilst NHERF1 is associated with the ependymal cells. We propose that NHERFs1 and 2 may play distinct roles in regulating CSF formation. Further roles for NHERFs 1 and 2 are possible; both have PDZ binding sites; thus in brain astrocytes NHERF1 anchors the glutamate transporter GLAST which has a PDZ motif. However in the choroid plexus glutamate transporters such as GLAST appear to be absent. It is possible that other PDZ motif-containing proteins such as aquaporins may be anchored by the NHERFs, but this awaits further study.

## POS-MON-239

## VOLUNTARY RUNNING REVERSES AGE-ASSOCIATED COGNITIVE DECLINE IN THE PLACE TASK OF THE AGED RODENT

Siette J.<sup>1</sup>, Westbrook R.F.<sup>1</sup>, Sachdev P.<sup>2,3</sup> and Valenzuela M.<sup>2,3</sup><sup>1</sup>School of Psychology, University of New South Wales, Sydney Australia. <sup>2</sup>School of Psychiatry, University of New South Wales, Sydney Australia. <sup>3</sup>Neuropsychiatric Institute, Prince of Wales Hospital, Sydney Australia.

**Background.** There is a potential role for neurogenesis in restoring neuronal and synaptic loss, major pathological features of many disorders associated with ageing, including Alzheimer's Disease, Parkinson's Disease, Motor Neuron Disease and other disorders. Voluntary motor activity may constitute one of the triggers for neurogenesis. The aims of this study were to: 1) identify the most sensitive behavioural measure of age-associated cognitive performance in rodents; and 2) assess whether voluntary wheel running reduce the adverse effects of ageing on cognitive performance. **Method.** Aged (18 months) and young (7 weeks) female Fischer rats had free access to a running wheel for 8 weeks. Animals were exposed to four different behavioural paradigms: the Object vs Place Task, the Morris Water Maze Task and the Localized Cue Task. **Results.** Results indicate that the Object vs Place Task is a valid behavioural measure for future assessments of brain regeneration in rodent studies. Specifically, aged rats (n = 7) are significantly impaired at the PLACE task (a hippocampal-dependent measure) but not at the OBJECT task (a hippocampal-independent measure). They also show that voluntary running is able to selectively reverse PLACE task performance and spare OBJECT task performance. **Conclusions.** Future research examining neuro-restorative treatments should consider this task in their measures of recovered cognitive function.

## POS-MON-240

## EVIDENCE FOR A NEW "LOAD AND LOCK" MODEL OF THE TRKA AND P75NTR HIGH-AFFINITY NGF RECEPTOR COMPLEX

Sykes A.M.<sup>1</sup>, Abankwa D.<sup>2</sup>, Palstra N.<sup>1</sup>, Hancock J.F.<sup>2</sup> and Coulson E.J.<sup>1</sup><sup>1</sup>Queensland Brain Institute, The University of Queensland, Brisbane 4072 QLD. <sup>2</sup>Institute for Molecular Bioscience, The University of Queensland, Brisbane 4072 QLD.

The neurotrophin receptors, p75 neurotrophin receptor (p75NTR) and TrkA, form a high-affinity complex that mediates the fundamental trophic actions of their ligand, nerve growth factor (NGF), both during development and in the adult nervous system. Here we show that NGF neuronal survival signaling through this high-affinity complex requires the proteolytic generation of a C-terminal fragment of p75NTR lacking its extracellular ligand-binding and transmembrane domains. This p75NTR γ-secretase cleavage fragment physically associates with TrkA through an eight amino acid juxtamembrane domain but, coincident with TrkA signaling, fluorescence resonance energy transfer (FRET) analyses indicate that the p75NTR fragments are disengaged from their otherwise constitutively self-associated state. We propose that the high-affinity NGF-receptor complex comprises a TrkA dimer pillared by the four p75NTR intracellular domain fragment monomers; these fragments act to lock the bound NGF to TrkA, thereby increasing receptor affinity and facilitating TrkA survival signaling.



## POS-TUE-241

### EVIDENCE FOR Q<sub>0</sub> SITE OF MITOCHONDRIAL COMPLEX III AS THE SOURCE OF INCREASED PRODUCTION OF SUPEROXIDE IN CARDIAC MYOCYTES AFTER TRANSIENT EXPOSURE TO HYDROGEN PEROXIDE

Viola H.M.<sup>1</sup>, Ingley E.<sup>2</sup> and Hool L.C.<sup>1</sup>

<sup>1</sup>School of Biomedical Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009. <sup>2</sup>The Western Australian Institute for Medical Research, Nedlands, WA 6009.

Oxidative stress is a feature of cardiovascular disease. We have previously shown that exposure of adult guinea-pig ventricular myocytes to 30µM hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 5min results in increased mitochondrial superoxide production. This causes a 2-fold increase in protein synthesis, suggesting transient exposure to H<sub>2</sub>O<sub>2</sub> may be sufficient to induce cardiac hypertrophy in cardiac myocytes. Previous results suggested the source of superoxide was distal to complex I. We performed additional experiments to further explore the site of superoxide production. We exposed myocytes to 7nM myxothiazol that binds at complex III Q<sub>0</sub> ROS generation site and examined superoxide generation assessed as changes in dihydroethidium (DHE) fluorescence after exposing myocytes to 30µM H<sub>2</sub>O<sub>2</sub> for 5min then 10U/ml catalase for 5min. Myxothiazol completely attenuated the increase in DHE signal (n=16, p<0.05). In addition 7nM stigmatellin that also binds at complex III Q<sub>0</sub> ROS generation site attenuated the DHE signal 63% (n=5, p<0.05). However, exposing myocytes to 7nM antimycin A that binds at complex III Q<sub>1</sub> ROS generation site did not alter the DHE signal after exposure to 30µM H<sub>2</sub>O<sub>2</sub>. These data suggest the source of ROS production after transient exposure to H<sub>2</sub>O<sub>2</sub> is the Q<sub>0</sub> site of complex III. We have confirmed the results by assessing changes in DHE fluorescence in the myocytes in the presence of mitochondrial complex substrates administered via the patch-pipette. Complex III may represent a possible site to target in the prevention of the development of cardiac hypertrophy associated with oxidative stress.

## POS-TUE-243

### GABA<sub>A</sub> RECEPTORS INCREASE THEIR CONDUCTANCE THROUGH NOVEL PROTEIN INTERACTIONS

Tierney M.L.<sup>1</sup>, Everitt A.B.<sup>1</sup>, Seymour V.A.L.<sup>1</sup>, Curmi J.<sup>1</sup> and Laver D.R.<sup>2</sup>

<sup>1</sup>JCSMR, Australian National University. <sup>2</sup>School of Biomedical Science, University of Newcastle.

Native GABA<sub>A</sub> channels display a single-channel conductance ranging between ~10-90 pS. Diazepam increases the conductance of some of these native channels but never those of recombinant receptors unless they are co-expressed with GABARAP. This trafficking protein clusters recombinant receptors in the membrane suggesting that high-conductance channels arise from receptors that are at locally high concentrations. The amphipathic (MA) helix that is present in the large cytoplasmic loop of every subunit of all ligand-gated ion channels mediates protein-protein interactions. Here we report that when applied to inside-out patches, a peptide mimicking the MA helix of the γ2 subunit (γ381-403) of the GABA<sub>A</sub> receptor abrogates the potentiating effect of diazepam on both endogenous receptors and recombinant GABA<sub>A</sub> receptors co-expressed with GABARAP, by substantially reducing their conductance. The protein interaction disrupted by the peptide did not involve GABARAP because a shorter peptide (γ386-403) known to compete with the γ2: GABARAP interaction did not affect the conductance of recombinant αβγ receptors co-expressed with GABARAP. The requirement for receptor clustering and the fact that the γ2 MA helix is able to self-associate support a mechanism whereby adjacent GABA<sub>A</sub> receptors interact via their γ2 subunit MA helices, altering ion permeation through each channel. This finding has important implications for understanding both the structural design of ligand-gated ion channels and the adaptive, dynamic means a cell invokes to amplify its signalling capacity.

## POS-TUE-242

### AN IMPROVED OPEN CHANNEL STRUCTURE OF MSL

Corry B.<sup>1</sup>, Hurst A.C.<sup>2</sup>, Pal P.<sup>1,2,3</sup>, Rigby P.<sup>1</sup> and Martinac B.<sup>2,3,4</sup>

<sup>1</sup>The University of Western Australia. <sup>2</sup>The University of Queensland.

<sup>3</sup>Victor Chang Cardiac Research Institute. <sup>4</sup>The University of New South Wales.

Mechanosensitive channels act as molecular transducers of mechanical force exerted on the membrane of living cells by opening in response to membrane bilayer deformations occurring in physiological processes such as touch, hearing, blood pressure regulation and osmoregulation. Here, we determine the likely structure of the open state of the mechanosensitive channel of large conductance (MscL) using a combination of patch-clamp, FRET spectroscopy, data from previous EPR experiments and molecular and Brownian dynamics simulations. In our method, structural rearrangements of the protein can be measured in similar conditions as patch clamp recordings while controlling the state of the pore in its natural lipid environment by modifying lipid bilayer morphology. Transition to the open state is less dramatic than previously proposed, while the N-terminus is seen to be able to directly translate membrane tension to the conformation of the pore lining helix. Combining FRET data obtained in physiological conditions with simulations is likely to be of great value for studying conformational changes in a range of multimeric membrane proteins.

## POS-TUE-244

### L-DOPA IS INCORPORATED INTO PROTEINS BY DOPAMINERGIC NEURONES AND CAN CAUSE APOPTOTIC CELL DEATH

Wang S. and Rodgers K.

The Heart Research Institute, 7 Eliza Street Newtown, NSW, Australia.

L-DOPA (levodopa), the direct precursor of dopamine in dopaminergic neurones, is the most widely used treatment for Parkinson's disease (PD). It remains uncertain as to whether L-DOPA is neurotoxic and accelerates the progression of PD. A potential mechanism of L-DOPA neurotoxicity has been overlooked; L-DOPA is a close structural analogue of the amino acid tyrosine, and can become misincorporated into proteins by protein synthesis. In the present studies we show for the first time that L-DOPA is misincorporated into proteins by dopaminergic neuronal cells (SH-SY5Y) at concentrations of L-DOPA (1µM) reported in the cerebrospinal fluid of L-DOPA-treated PD patients. In support of this we demonstrate that DOPA-containing proteins are elevated in the brains of rats (n>5) and humans (n>5) treated with L-DOPA. DOPA-containing proteins can resist proteolysis. SH-SY5Y cells incubated with L-DOPA accumulate autofluorescent perinuclear protein aggregates. We show, using a range of apoptosis assays (Annexin V binding, caspase 3 activation, COMET assay), that proteins containing incorporated DOPA trigger apoptosis in dopaminergic neurones in vitro suggesting that a similar mechanism could accelerate neuronal loss in vivo. To further explore the role of L-DOPA misincorporation into proteins in PD we investigate L-DOPA incorporation into alpha-synuclein, a protein associated with PD pathogenesis and progression. Using SH-SY5Y cells that over-express alpha-synuclein we demonstrate that incorporation of L-DOPA increases alpha-synuclein aggregation and toxicity. Incorporation of L-DOPA into proteins could increase the rate of neurodegeneration in PD patients.

## POS-MON-245

## REDUCED THEORETICAL ESTIMATES OF THE ELECTROTONIC LENGTH CONSTANT FOR NEUROPROSTHETIC ELECTRICAL STIMULATION

Meffin H.<sup>1,2</sup> and Kamenewa T.<sup>1,2</sup><sup>1</sup>NICTA. <sup>2</sup>The University of Melbourne.

The electrotonic length constant ( $\lambda$ ) of a dendrite determines the spatial scale over which a localized input propagates passively to neighbouring portions of a dendritic tree. It arises as a parameter in the cable equation. Theoretical estimates for  $\lambda$  are in the range of hundreds of micrometers, depending on neural type. However, two key assumptions underlying these estimates breakdown under conditions relevant to electrical stimulation by neuroprostheses. These assumptions are: (1) extracellular equipotentiality, or equivalently, negligible extracellular resistance per unit length of dendrite ( $r$ ); (2) steady state conditions. In fact, extracellular stimulation produces a non-equipotential field and  $r$  is large due to the extremely confined extracellular space. Also the duration of neuroprosthetic stimulation is very brief (10-1000  $\mu$ s) compared to the time-scale of most intrinsic events ( $> 1$  ms). A revised model is described that incorporates these two conditions as relevant to neuroprosthetic stimulation. The cable equation is derived as a good approximation to the problem with full three-dimensional geometry. A new expression for  $\lambda$  is given that applies to conditions relevant to neuroprosthetic stimulation. It predicts that the length constant depends on the duration of the stimulus pulse, but is largely independent of neural type. Estimates of  $\lambda$  range from 3 to 30  $\mu$ m, which is between one and three orders of magnitude smaller than conventional estimates from the literature. The revised estimate is small compared to the extent of most dendritic trees. The predicted consequence of this is that during neuroprosthetic stimulation, the passive depolarization of a dendritic section is determined by the local extracellular current density, but is unaffected by the propagation of potentials from dendritic sections more than a few tens of microns away.

## POS-MON-246

## ADULT CANINE NEUROGENESIS: A DORSAL VERSUS VENTRAL HIPPOCAMPAL GRADIENT?

Lowe A.K.<sup>1,4</sup>, Dalton M.A.<sup>2</sup>, Sachdev P.<sup>1,3,4</sup>, Sidhu K.S.<sup>1,4</sup> and Valenzuela M.J.<sup>1,3,4</sup><sup>1</sup>School of Psychiatry, The University of New South Wales, Sydney, Australia.<sup>2</sup>Prince of Wales Medical Research Facility, Prince of Wales Hospital, Sydney, Australia. <sup>3</sup>Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia.<sup>4</sup>Stem Cell Laboratory, Faculty of Medicine, The University of New South Wales, Sydney, Australia.

**INTRODUCTION:** Neurogenesis has been observed in the hippocampus of numerous adult mammalian species as assessed by immunohistochemistry (IHC) and *in-vitro* assays of stem cell activity. The canine hippocampus is unique, however, as it contains two distinct dentate gyrus areas in the dorsal and ventral positions. This study aims to assess whether there is a neurogenic and stem cell activity gradient between these two regions of the adult canine hippocampus. **METHODS:** Whole hemisphere coronal sections of adult canine brain were immunohistochemically examined for Doublecortin (DCX). Dorsal and ventral hippocampal regions were also dissected from fresh canine brain, and cells dissociated and transferred to bulk neurosphere culture, limiting dilution assay, or colony forming assay to quantify proliferation potential. Polymerase Chain Reaction (PCR) analysis of neural markers, immunocytochemical staining and EDU proliferation studies were performed on early passage cells to further assess cell potential. **RESULTS:** The dorsal region of the canine hippocampus had a much higher density of DCX positive cells than the ventral region under *post mortem* IHC analysis. Cells from both regions were capable of proliferating to form neurospheres *in-vitro* after primary passage, thus indicating the presence of stem or progenitor cells. These findings were supported by subsequent PCR and immunocytochemical analysis of neural stem cell markers. The colony forming assay revealed a significantly larger number of spheres  $> 10\mu$ m in the Dorsal Hippocampus ( $p < 0.05$ ), while EDU proliferation studies ( $n=3$ ) also support a dorsal-ventral gradient. These results, however, were not supported by the limiting dilution assay which indicated identical colony forming ability of both dorsal and ventral hippocampal regions (1 cell in 572). **CONCLUSIONS:** Immunohistochemical analysis suggests enhanced neurogenesis in the dorsal region of the hippocampus compared to the ventral region in the adult canine brain on the basis of DCX, a marker of immature migrating neurons. A dorsal-ventral hippocampal gradient was observed *in-vitro* using various techniques. The increased proliferation rates of dorsal hippocampal derived cells compared to ventral derived *in-vitro* suggest a higher density of precursor cells present in the dorsal hippocampus.

## POS-MON-247

## CONTRIBUTION OF NMDA RECEPTORS TO SYNAPTIC TRANSMISSION IN LAYER 5 OF THE MEDIAL PREFRONTAL CORTEX

Marek R. and Faber E.S.L.

The University of Queensland, Queensland Brain Institute, Brisbane, QLD 4072, Australia.

**Introduction:** The medial prefrontal cortex (mPFC) is fundamentally involved in mediating higher cognitive tasks such as working memory. During working memory tasks neurons in the mPFC fire repetitively. This firing is thought to be maintained in part by synaptic reverberation in layer 5 (L5) pyramidal neuron networks, and has been proposed to be dependent on NMDA receptor activation (Wang, 2001). In this study we investigated the contribution of NMDA receptors to synaptic transmission in L5 pyramidal neurons. **Methods:** Coronal brain slices (300  $\mu$ m) containing mPFC neurons were cut from P18–P29 Wistar rats (of either sex). Paired whole-cell recordings were made from either L5-L5 pairs of neurons or L5-L2/3 pairs, with a potassium-based internal solution. Synaptic responses were evoked by giving suprathreshold depolarizing current injections in the presynaptic neuron. **Results:** The rate of connections between L5-L5 pairs was 7% ( $n=18/272$ ) and 13% ( $n=6/45$ ) at L5-L2/3 pairs. In the presence of apamin (100 nM, to block SK channels) the NMDA receptor antagonist AP5 (30  $\mu$ M) reduced the EPSP amplitude at L5-L5 pairs to  $59 \pm 15\%$  of control ( $n=8$ ,  $p < 0.05$ ). In contrast at L2/3-L5 pairs AP5 reduced EPSPs to  $79 \pm 3\%$  of control ( $n=6$ ,  $p < 0.05$ ). **Conclusion:** Our results show that NMDA receptors make a substantial contribution to synaptic transmission in L5 pyramidal neurons in the mPFC, with a trend for a larger contribution at L5-L5 synapses than L2/3-L5 synapses. This contribution is masked by opposing synaptic SK channel activity. These data support the proposal that there is a large NMDA receptor activation at L5 synapses that contributes to sustained activity in L5 networks observed during working memory tasks.

## POS-MON-248

## LINKING THE BRAIN AND HEART: ALTERATIONS IN CARDIAC FUNCTION AND HCN CHANNEL EXPRESSION IN GENETICALLY EPILEPTIC RATS

Ng C.<sup>1</sup>, Urmaliya V.<sup>2</sup>, Powell K.L.<sup>1</sup>, Kennard J.T.T.<sup>1</sup>, Jones N.C.<sup>1</sup>, Megatia I.<sup>1</sup>, Pinault D.<sup>3</sup>, White P.<sup>2</sup> and O'Brien T.J.<sup>1</sup><sup>1</sup>Department of Medicine, Royal Melbourne Hospital, University of Melbourne, AUSTRALIA. <sup>2</sup>Department of Pharmaceutical Biology,Monash University, AUSTRALIA. <sup>3</sup>Faculte de Medecine, Universite de Strasbourg, FRANCE.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels play an important role in the generation of pacemaker activity in the brain and heart. Several human and animal epilepsy studies have reported alterations in HCN expression and function in the brain. Epilepsy is associated with an increased risk of sudden unexplained death (SUDEP), possibly due to cardiac arrhythmias and changes in HCN channels may be the underlying molecular mechanism. Here, we have examined cardiac electrophysiology expression of HCN channel subunits in the hearts of Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a widely used animal model of absence epilepsy. HCN mRNA and protein expression in cardiac chambers of GAERS and non-epileptic control rats (NEC) was assessed using qPCR and Western blot. Electrocardiograms were recorded in anaesthetized rats, and in isolated heart preparations. HCN2 and HCN4 mRNA expression was significantly decreased ( $p < 0.05$ ) in GAERS ( $n=8-10$ ) compared to NEC ( $n=8-10$ ) rats, and HCN1 protein expression was significantly decreased ( $p < 0.0005$ ). Consistent with these data, cardiac function was significantly altered ( $p < 0.05$ ) *in-vivo* in GAERS ( $n=10$ ) with shorter QRS duration, slower heart rate and greater standard deviation of RR intervals (indicative of cardiac dysrhythmia) compared to NEC rats ( $n=5$ ). These findings were replicated in the isolated heart preparations ( $n=10$  for both strains), and overall are suggestive of a mechanistic link between alterations in ion channel expression in the heart and brain, and may contribute to the increased risk of SUDEP.

## POS-MON-249

**SYNAPTIC ORGANIZATION OF MSO PRINCIPAL NEURONS IN HEARING, DEAF AND COCHLEAR-IMPLANTED CATS**

Tirko N.N., Pongstaporn T. and **Ryugo D.K.**  
Johns Hopkins University, Baltimore, MD 21205 USA.

The medial superior olive (MSO) is a key component of the central auditory pathway that has been implicated in the processing of interaural time differences (ITDs) used for sound localization. The deaf white cat is a proven animal model of congenital deafness and was utilized to examine how deafness and cochlear implantation affected the synaptic organization of MSO principal neurons. Synaptic inputs to the MSO were investigated using electron microscopic examination of postsynaptic density characteristics as well as an objective method for synaptic vesicle (SV) analysis in which synapses were classified as excitatory or inhibitory based on a quantitative assessment of SV size and roundness. Analysis revealed that the principal neurons in the MSO of deaf cats (n=2) have a decreased number of axosomatic inhibitory endings and axodendritic excitatory endings as compared to normal hearing cats (n=2). To examine the possible restorative effects of cochlear implant stimulation on the MSO, congenitally deaf cats aged 3 months (n=4) received unilateral cochlear implants that had been modified by Advanced Bionics Corporation for use in kittens. Implantation and stimulation strategies were virtually identical to those used for human recipients. Animals were stimulated 5 days a week over a period of 3 months. Results show that MSO principal neurons from the cochlear implant animals have an increased number of axosomatic inhibitory inputs as compared to deaf cats. There is also a return of excitatory inputs to MSO dendrites. These results support the hypothesis that early cochlear implantation can induce synaptic plasticity to restore auditory neurons to a more normal state.

## POS-MON-250

 **$\alpha$ 4/7-CONOTOXIN REGIIA SELECTIVELY TARGETS THE  $\alpha$ 3 $\beta$ 2 NACHR**

Franco A.<sup>1</sup>, Nevin S.<sup>2</sup>, Akondi K.<sup>3</sup>, Melaun C.<sup>4</sup>, Daly N.<sup>3</sup>, Luetje C.<sup>5</sup>, Alewood P.<sup>3</sup>, Craik D.<sup>3</sup>, Adams D.<sup>2</sup> and **Mari F.<sup>1</sup>**

<sup>1</sup>Department of Chemistry & Biochemistry, Florida Atlantic University, Boca Raton, Florida, USA. <sup>2</sup>Health Innovations Research Institute, RMIT University, Bundoora, VIC 3083, Australia. <sup>3</sup>IMB, University of Queensland, Brisbane, QLD 4072, Australia. <sup>4</sup>Justus Liebig Universität Giessen, Institut für Allg. Zoologie und Entwicklungsbiologie, Giessen, Germany. <sup>5</sup>Department of Molecular & Cellular Pharmacology, Miller School of Medicine, University of Miami, Miami, Florida 33101, USA.

Neuronal nicotinic acetylcholine receptors (nAChRs) play an important role in the central and peripheral nervous system and may have important implications in certain disease states including Parkinson's disease and schizophrenia. Structurally, they are either homopentamers such as  $\alpha$ 7 nAChRs, or heteropentamers of  $\alpha$ 2-6 and  $\beta$ 2-6 subunits.  $\alpha$ 9 $\alpha$ 10 nAChRs are unusual heteromeric receptors that are composed of only alpha subunits.  $\alpha$ -conotoxins are peptides isolated from the venom of cone snails that are constrained by two disulphide bonds (1-3, 2-4).  $\alpha$ -conotoxins inhibit nAChRs, targeting diverse receptor subtypes. One subfamily of these  $\alpha$ -conotoxins, the  $\alpha$ 4/7-subfamily, has a characteristic -CCX4CX7C- motif, and is the largest subclass of  $\alpha$ -conotoxins that target neuronal nAChRs. Using size exclusion and reversed phase HPLC we isolated nanomolar quantities of a novel  $\alpha$ 4/7-conotoxin (RgIIA). RgIIA was isolated from the venom of *Conus regius*, a worm-hunting species from the Western Atlantic Ocean. Its sequence was directly determined by Edman degradation and confirmed by the cDNA sequence of its protein precursor, which also indicates that RgIIA belongs to the A-superfamily of conotoxins. nanoNMR of native RgIIA shows the chemical shift dispersion characteristic of a folded  $\alpha$ -conotoxin. Milimolar quantities of RgIIA were produced using solid phase peptide synthesis based on Boc chemistry. Native and synthetic RgIIA samples were functionally tested using voltage clamp electrophysiology on nAChRs expressed in *Xenopus laevis* oocytes. RgIIA is a selective inhibitor of the  $\alpha$ 3 $\beta$ 2 nAChRs (n=3), and its potency surpasses MII, the most selective and potent  $\alpha$ -conotoxin known to target the  $\alpha$ 3 $\beta$ 2 nAChR. RgIIA exhibits sequence differences when compared to the  $\alpha$ 3 $\beta$ 2 subunit-specific  $\alpha$ 4/7-conotoxins such as MII and PnIA isolated from a fish-hunting and mollusk-hunting cone snails, respectively. Thus, while structurally related to other  $\alpha$ 4/7 conotoxins, RgIIA has an exquisite balance of shape, charges, and polarity exposed on its structure to selectively and potently block the  $\alpha$ 3 $\beta$ 2 nAChR.

## POS-MON-251

**GLYCATED PROTEINS INHIBIT K<sup>+</sup> CHANNELS IN ISO-LATED VASCULAR SMOOTH MUSCLE CELLS**

Yang Y.<sup>1</sup>, de Dios S.<sup>3</sup>, Jenkins A.J.<sup>3</sup>, Davis, M.J.<sup>1,2</sup> and **Hill M.A.<sup>1,2</sup>**  
<sup>1</sup>Dalton Cardiovascular Research Center and Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri, USA. <sup>2</sup>Department of Medicine, University of Melbourne, <sup>3</sup>St Vincent's Hospital, Fitzroy, Victoria.

Fibronectin (FN) has been shown to enhance K<sup>+</sup> channel activity via an integrin-mediated mechanism. As vascular smooth muscle (VSM) K<sup>+</sup> channels mediate vasodilation, we investigated whether advanced glycation of fibronectin (as occurs in diabetes and renal failure) alters the normal stimulatory effect of this matrix protein on these channels. Under sterile conditions, FN (1mg/ml) was glycated (gFN) for 5 days in the presence of methylglyoxal (50mM) or glycolaldehyde (50mM). Albumin, a non-matrix protein, was similarly glycated as a control. VSM cells were enzymatically isolated from rat cerebral arteries for K<sup>+</sup> channel activity studies via whole cell patch clamp. The inhibitors, iberiotoxin (0.1 $\mu$ M) and 4-aminopyridine (1mM), were used to identify contributions of large conductance, Ca<sup>2+</sup>-activated, K<sup>+</sup> channels and voltage-gated K<sup>+</sup> channels, respectively. While native FN enhanced whole cell K<sup>+</sup> current (1.8 fold), gFN caused a 56% inhibition of current compared to baseline. Furthermore, native albumin did not enhance basal K<sup>+</sup> current but when glycated caused inhibition (61%; p < 0.05). Inhibitor studies indicated a predominant effect of gFN on the Kv component of total K<sup>+</sup> current. These studies provide a potential mechanism by which advanced glycated proteins impair VSM function and adversely impact arteriolar vasodilation.



## POS-TUE-001

**PHYSICAL EXERCISE ACTIVATES ENDOGENOUS NEURAL PRECURSOR CELLS FOLLOWING IRRADIATION OF THE AGING MURINE BRAIN**

**Blackmore D.G.<sup>1</sup>**, Rietze R.L.<sup>2</sup>, Waters M.J.<sup>1</sup> and Bartlett P.F.<sup>1</sup>  
<sup>1</sup>Queensland Brain Institute, University of Queensland. <sup>2</sup>Pfizer Regenerative Medicine, Cambridge, United Kingdom.

Given the rapid increase in age-related neurodegenerative diseases, including dementia, it is of critical importance to identify strategies to slow, prevent or even reverse age-associated cognitive decline. Although it is well established that normal aging correlates with decreases in neurogenesis and cognitive function, little is currently known regarding the effects of aging on the endogenous neural precursor cell population. We recently revealed that within the ventricular neuraxis of the mouse there is a decrease in the number of neural precursor cells with age. This decrease was evident at 6 months (~40% decrease), culminating in an approximate 90% decrease at 24 months of age. Given our recent finding that physical exercise can stimulate precursor cells within the subventricular zone (SVZ) we sought to investigate if voluntary exercise can augment the regenerative capacity of the aging SVZ. Utilising a single dose of irradiation (3.5Gy) to ablate dividing cells we assessed the regenerative capacity of the SVZ in 12m old animals. In non-running animals neurosphere numbers decreased from naive levels (905±65, N=3), resulting in incomplete repopulation of the SVZ at both one and two weeks (597±14 and 528±19 respectively, N=3) post-ablation. This is in sharp contrast to animals that were provided access to a running wheel. In these animals neurosphere numbers in the SVZ were restored to naive levels within one week (935±39, N=3). Similar results were also obtained for 18m old animals, demonstrating that physical exercise following ablation/injury is able to augment regenerative capacity within the aged brain. Studies are currently focusing on the potential mechanisms involved in the activation of these endogenous precursor cells.

## POS-TUE-002

**TROPOMYOSIN ISOFORMS DIFFERENTIALLY IMPACT ON THE BRANCHING OF PROCESSES IN DIFFERENTIATED B35 NEUROBLASTOMA CELLS**

**Fath T.<sup>1,2</sup>**, Bryce N.<sup>2</sup>, Connor A.<sup>3</sup>, Schvezov G.<sup>2,3</sup> and Gunning P.W.<sup>2,3</sup>  
<sup>1</sup>School of Medical Sciences, Department of Anatomy, University of New South Wales, Sydney. <sup>2</sup>Discipline of Paediatrics and Child Health, University of Sydney, Sydney. <sup>3</sup>School of Medical Sciences, Department of Pharmacology, University of New South Wales, Sydney.

During early neuronal development the actin cytoskeleton is instrumental to support morphological changes. Tropomyosin proteins are a family of proteins that associate with and regulate the dynamics and function of actin filaments. The association of tropomyosins with the actin filaments can either prevent or facilitate, in an isoform dependent manner, the access of a number of actin-associated proteins. In eukaryotic cells more than 40 isoforms are generated by alternative splicing from four different genes ( $\alpha$ -,  $\gamma$ - and  $\delta$ -gene) which are developmentally and spatially regulated. Products from three of the tropomyosin genes, the  $\alpha$ - (Tm5a/b, TmBr1, TmBr2, TmBr3)  $\gamma$ - (Tm5NM1-11) and  $\delta$ -tropomyosin (Tm4) gene, are found in neurons. We have recently shown that increased levels of Tm5NM1 result in an extension of neurite length and degree of branching. We have now investigated the effect of increased protein levels of the brain specific isoforms TmBr1-3, on process formation using the B35 neuroblastoma derived cell line. We show that these isoforms differentially impact on the branching pattern of processes. While increased levels of TmBr1 result in almost complete elimination of branching, overexpression of TmBr2 and TmBr3 leads to more branching as compared to control (n=3). Our results demonstrate that the actin cytoskeleton has a critical role in generating a specific branching pattern during process outgrowth. This suggests an important role for tropomyosins when neurons establish their complex network of highly branched neurites.

## POS-TUE-003

*Cancelled*

## POS-TUE-004

**THE ROLE OF NEDD4-2 IN NEURITE DEVELOPMENT**

**Donovan P.J.<sup>1</sup>** and Poronnik P.<sup>2</sup>

<sup>1</sup>School of Biomedical Sciences Skerman Building (65) The University of Queensland St Lucia QLD 4072. <sup>2</sup>School of Medical Sciences and Health Innovations Research Institute; RMIT, Australia.

Nedd4-2 is an ubiquitin protein ligase that binds to the consensus motif L/PPXY in target proteins and catalyses the transfer of ubiquitin, thereby regulating protein turnover and degradation. Nedd4-2 is a developmentally regulated transcript in the mouse brain and has recently been identified as regulator of a number of neuronal receptors and transporters. We have found that silencing of Nedd4-2 results in an increased number of neurites per cell in differentiated PC12 cultures and that when Nedd4-2 is over expressed there is a 50 %  $\pm$  4 (p<0.001; n=4) reduction in differentiation efficiency. We hypothesized this is due to Nedd4-2 ubiquitinating neurogenic proteins involved in neurite outgrowth. An *in silico* screen for neurogenic proteins containing the Nedd4-2 recognition motif identified the Microtubule Associated Protein 2 (MAP2) as a putative binding candidate. We show here that Nedd4-2 binds to and co-localises with the high molecular weight isoform of MAP2. Additionally in the Nedd4-2 null mouse expression of MAP2 is increased in the embryonic brain. Preliminary data shows that MAP2 is also ubiquitinated in PC12 cells. Furthermore inhibition of the proteasome in these cells results in a 4-fold increase in the abundance of MAP2 compared with controls (< 0.0001; n=4). MAP2 is known to determine the availability of tubulin subunits for polymerization into microtubules. We report here that silencing of Nedd4-2 results in a 3-fold increase in both alpha and acetylated tubulin fluorescence density. These data suggest Nedd4-2 is a negative regulator and a rate-determining factor in neurite outgrowth due to its ability to sequester alpha tubulin subunits through its regulation and ubiquitination of MAP2.

## POS-TUE-005

## THE SERINE PROTEASE INHIBITOR NEUROSERPIN REGULATES DENDRITIC PROTRUSION NUMBER AND DENDRITIC SPINE MORPHOLOGY

**Borges V.M.**, Lee T.W., Christie D.L. and Birch N.P.  
School of Biological Science and the Centre for Brain Research, The University of Auckland, Auckland 1010, New Zealand.

It is thought that the cellular basis for learning and memory involves long-lasting changes at synapses occurring through morphological and functional plasticity. Dendritic spines are the postsynaptic components of most excitatory synapses in the brain. These dynamic structures are associated with plasticity of neural function as they are constantly changing in number and morphology throughout development and in the adult brain. Understanding the mechanisms of spine plasticity may provide insight to how learning and memory occurs at a cellular level. Neuroserpin, a member of the serpin superfamily, is principally expressed by neurons in the central and peripheral nervous system. Transgenic mice over-expressing neuroserpin show improved spatial learning, supporting a role for neuroserpin in synaptic plasticity. To investigate the cellular effect of neuroserpin on neuronal morphology, primary embryonic rat hippocampal neuronal cultures were transfected with a rat neuroserpin cDNA along with a plasmid expressing humanized *R. reniformis* GFP (hrGFP). In general, neurons overexpressing neuroserpin and hrGFP showed a similar global morphology to neurons expressing hrGFP alone. However, higher magnification images showed that there was an increase in dendritic protrusions in neuroserpin-overexpressing cells compared to controls ( $p < 0.001$ ). In addition the overexpression of neuroserpin altered dendritic spine morphology. Increased neuroserpin levels resulted in a 52% increase in the density of thin spines ( $p = 0.007$ ) and a 34% reduction in the density of mushroom spines ( $p = 0.03$ ). These results suggest an underlying mechanism for neuroserpin's effects on learning and memory.

## POS-TUE-007

TEN-M3 IS EXPRESSED IN MATCHING GRADIENTS IN THE DEVELOPING VISUAL SYSTEM OF THE WALLABY, *MACROPUS EUGENII*

**Carr O.P.**<sup>1</sup>, Glendining K.A.<sup>2</sup>, Leamey C.A.<sup>2</sup> and Marotte L.R.<sup>1</sup>  
<sup>1</sup>Visual Sciences Group, Research School of Biological Sciences, The Australian National University, Canberra, Australia. <sup>2</sup>Discipline of Physiology and Bosch Institute, University of Sydney, NSW, Australia.

**Purpose:** Ten-m3, a transmembrane glycoprotein, influences the mapping of ipsilaterally-projecting retinal axons to central targets in the mouse. The aim of this study was to determine the pattern of expression of Ten-m3 in the retina and superior colliculus (SC) of the Tammar wallaby during development of the retinocollicular projection. **Methods:** Real-time qualitative polymerase chain reaction for Ten-m3 mRNA was performed on whole RNA isolated from animals aged P15, P30, P45, P65, P95, and adults ( $n=4$  per group). Samples were compared between dorsal and ventral halves of the retina, and medial and lateral areas of the SC. *In situ* hybridisation for Ten-m3 mRNA was performed on cryosectioned retina and SC in animals aged P15, P30, and P45 ( $n=2$  per group). **Results:** Ten-m3 was detected in all age groups. In ages P15-P95, expression of Ten-m3 was significantly higher in ventral compared to dorsal retina, and medial compared to lateral SC (all groups;  $p < 0.05$ , Student's t-test). These differences were greatest at P30 (retina;  $p < 0.01$ , SC;  $p < 0.01$ , One-way ANOVA). At P15-P45, expression was in an increasing dorsoventral gradient in the ganglion cell layer of the retina and a decreasing mediolateral gradient in the retinorecipient layers of the SC. **Conclusion:** Ten-m3 is expressed during development of the retinocollicular projection and takes the form of matching gradients in the retina and SC: regions that connect with each other express Ten-m3 at similar relative levels. The wallaby's protracted *ex utero* development will allow manipulation of Ten-m3 at early developmental stages not attainable in placental mammals.

## POS-TUE-006

## SPATIAL LEARNING DEFICITS IN TEN\_M KNOCKOUT MICE

**Bourke M.D.**<sup>1</sup>, Haast R.<sup>1</sup>, Young T.R.<sup>1</sup>, Fassler R.<sup>2</sup>, Leamey C.A.<sup>1</sup> and Sawatari A.<sup>1</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences and the Bosch Institute, University of Sydney, Australia. <sup>2</sup>Department of Molecular Medicine, Max Planck Institute of Biochemistry, Martinsried, Germany.

**Purpose:** Internal and external connections of the hippocampal formation (HCF) are topographically organised. One important function of these brain regions is thought to be spatial learning. The maintenance of topographic relationships aids in the association of neural representations between related brain regions. The HCF and the areas it connects with express mRNA of each member of the Ten\_m family of transmembrane proteins in discrete but overlapping patterns during development. Studies from our lab indicate that Ten\_m2, 3, and 4 are functionally important axonal guidance molecules in the visual system *in vivo*. **Methods:** Performance in the hidden-platform Morris water maze was compared between wildtype (WT;  $n=12$ ) and Ten\_m2, 3, and 4 knockout (KO;  $n=11$ , 12, 11) groups. During 7 days of acquisition mice had to locate a hidden platform using environmental cues. On the 8th day, mice were probed by relocating the platform to the opposite quadrant. Escape latency was compared for all groups and swim trajectories were recorded. **Results:** All groups of mice acquired the task, however all KO groups performed significantly worse compared to their WT littermates ( $p < 0.05$ ). All groups of mice learned the spatial reversal probe ( $p < 0.05$ ), but there was no difference between WTs and KOs ( $p > 0.05$ ). Qualitative analysis suggested that groups navigate using different strategies. **Conclusion:** These data suggest that spatial learning is defective in Ten\_m KO groups, consistent with the suggestion that Ten\_m proteins contribute to the development of the circuitry underlying spatial learning.

## POS-TUE-008

## MECHANISMS UNDERLYING THE ROLE OF TEN-M3 IN VISUAL DEVELOPMENT

**Glendining K.A.**, Nguyen M., Dharmaratne N., Sawatari A. and Leamey C.A.

Discipline of Physiology, School of Medical Sciences and Bosch Institute, University of Sydney, NSW, 2006, Australia.

**Purpose:** Ten-m3 knockout (KO) mice display profound abnormalities in mapping of ipsilateral retinal projections to the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC) at maturity, which are associated with visual deficits. The aim of this study was to determine the potential mechanisms underlying this by examining the trajectory of retinal axons in Ten-m3 KO and wildtype (WT) mice during development, and to identify causal molecular mediators of the mismapping phenotype. **Methods:** Bulk-fill retinal cholera-toxin B injections were used to trace retinal axons during development at P0, P3, P6 and P10 ( $n=3$  per group). Expression of genes with established roles in development of the retinofugal pathway were examined using *in situ* hybridisation ( $n=2$  per group), realtime PCR on total RNA from P0 mice ( $n=3$  per group), and wholemount preparations of neonate SC ( $n=3$  per group) incubated with EphA and ephrinA-AP probes. **Results:** In Ten-m3 KOs ipsilateral retinal axons enter the ventrolateral corner of the dLGN, unlike WTs where axons remain confined to the optic tract until they reach the dorsal part of the nucleus. EphA receptor expression in the developing SC is disrupted in Ten-m3 KOs as revealed by lower ephrinA-AP binding, and reduced EphA7 mRNA levels. Realtime PCR confirmed EphA disruption in KOs: with downregulation of EphA7 (19%,  $p \leq 0.05$ , 35%,  $p \leq 0.05$ ), and EphA5 mRNA (21%,  $p \leq 0.05$ , 30%,  $p \leq 0.05$ ) in SC and retina, respectively. Zic4 mRNA expression was downregulated in KO SC (25%,  $p \leq 0.0001$ ). **Conclusion:** Retinofugal axon guidance is disrupted in Ten-m3 KO mice. Alterations in EphA expression pose a potential mechanism for the retinofugal mismapping phenotype.

## POS-TUE-009

**TEN\_M3: ROLES IN THE DEVELOPMENT OF THE RODENT STRIATUM**

**Tran H.**, Sawatari A. and Leamey C.A.

Discipline of Physiology, School of Medical Sciences and Bosch Institute, The University of Sydney, NSW, 2006.

Ten\_m3 is a transmembrane glycoprotein which regulates cell adhesion and axonal guidance. We found that Ten\_m3 has a patchy distribution within the striatum of neonatal mice. Comparison of the in situ hybridisation signal for Ten\_m3 and immunohistochemistry for the  $\mu$ -opioid receptor ( $\mu$ OR1, a striosomal marker) suggests that Ten\_m3 is expressed in a subregion of the matrix. A potential role for Ten\_m3 in the compartmentalisation of the striatum into the striosomes and matrix was investigated using double staining for Wisteria floribunda agglutinin (WFA) and  $\mu$ OR1, in P7 and P21 wild type (WT) and Ten\_m3 knock out (KO) mice. Preliminary evidence indicates that a previously described transition in WFA labeling occurs identically in the two groups (n=3), suggesting that striatal compartments develop largely normally in Ten\_m3 KOs. To examine a potential role for Ten\_m3 in controlling the targeting of thalamostriatal axons, one of the major sources of input to the striatum, stereotaxic injections of biotinylated dextran amine were made into the parafascicular thalamic nucleus in adult mice. The area occupied by parafascicular terminals was increased in KOs, although this change was not significant (p=0.116, t-test). Interestingly, however, terminals were significantly denser in KOs than in WTs (WT:  $125.36 \pm 12.82$  (arbitrary units, mean  $\pm$  SEM), n = 6; KO:  $166.66 \pm 11.58$  (arbitrary units, mean  $\pm$  SEM), n = 5; p = 0.040, t-test). Qualitatively, this change was associated with a more uniform distribution of terminals in KOs, compared to a more "patchy" arrangement in WTs. This study suggests a novel role for Ten\_m3 in regulating the targeting of thalamostriatal projections to a specific subregion of the matrix.

## POS-TUE-010

**SEMAPHORIN3A IS INVOLVED IN THE AREAL DEVELOPMENT OF THE NONHUMAN PRIMATE VISUAL CORTEX**

**Homman-Ludiye J.** and Bourne J.A.

Australian Regenerative Medicine Institute, Monash University, Clayton, VIC 3800, Australia.

The nonhuman primate comprises multiple visual areas that have specific cytological boundaries, and that develop in an inside-out manner. However, the spatiotemporal mechanism by which these specific nuclei develop is poorly understood. For this present study, we demonstrate the role of the secreted guidance cue Semaphorin3A (Sema3A), a factor which has previously been demonstrated to guide the migration of newborn neurons in the cortex. Sema3A influences the polarisation of pyramidal neurons by inducing the axonal growth cone collapse and the attraction of the apical dendrite. In the developing marmoset monkey (*Callithrix jacchus*), we have established the immunohistochemical expression pattern of Sema3A from the end of cortical lamination (E130) until P14 (n=5), a temporal stage when the primary visual cortex (V1), second visual area (V2) and the middle temporal area (MT) are mature. At E130, Sema3A is expressed across the 6 layers of the visual cortex, except in V1 where Sema3A is restricted around NeuN<sup>+</sup> cell bodies located in layers 5 and 2. At later stages (P7), as V2 and MT mature, Sema3A expression in MT is localised around NeuN<sup>+</sup> cell bodies located in infra- and supra-granular layers as observed in V1 at E130. In V2, Sema3A is expressed around cell bodies only in infragranular layers and is still extensively expressed throughout the extracellular matrix of the subgranular layers. At P14, Sema3A overall expression decreases and is restricted around cell bodies. As maturation occurs in V1, MT then V2, Sema3A expression evolves from an extracellular to a pericellular pattern, in the infragranular prior to the subgranular layers. Our results suggest that Sema3A plays a role in controlling the spatial and temporal development of visual cortical areas, in addition to its role in cortical lamination. Furthermore, it is one of the specific factors involved in the early maturation of area MT at the same temporal stage as V1.

## POS-TUE-011

**STRUCTURAL CHANGES IN DENDRITIC ARBORS OF STRIATAL MEDIUM SPINY NEURONS COINCIDES WITH KEY POSTNATAL DEVELOPMENTAL EVENTS IN THE MOUSE**

**Lee H.** and Sawatari A.

Discipline of Physiology & Bosch Institute, University of Sydney, Sydney, NSW 2006.

Medium spiny neurons (MSN), the main output cells of the striatum, integrate corticostriatal and nigrostriatal inputs, thus mediating motor learning and reward-associated behaviours. Their maturation is considered vital for the establishment of basal ganglia function. Of key interest is a developmental period when major changes occur in neostriatal circuitry at around the second postnatal week, which correlates with the emergence of motor behaviours. Whilst increases in the number of spines and changes in the electrophysiological properties of MSNs within this developmental window have been reported previously, the gross changes occurring in dendritic morphology are less known. We used blind whole cell patch clamp technique to fill individual MSNs with neurobiotin in brain slices obtained from C57BL/6J mice at postnatal day (P)6-7, P9-11, P13-14, P21, P28-30, and P40+ (adult), then reconstructed 64 MSNs (6-10 from each age group).  $\mu$ -opioid receptor immunohistochemistry was performed on these same sections to localize the MSNs to either the matrix or striosomes. Among matrix MSNs, we found a significant increase in the mean length of dendrites between p6-7 and p9-12 and older (154-190%), despite a significant decrease (27-50%) in the number of branch points (p<0.05, post-hoc multiple-comparisons test). These data suggest both pruning and growth processes lead to the dramatic dendritic restructuring of these important neurons during a time in development that coincides with major circuitry and behavioural changes linked to basal ganglia function.

## POS-TUE-012

**STIM1 REGULATES SOCE, AND IS NECESSARY FOR GROWTH CONE TURNING**

**Mitchell C.B.**, Gasperini R., Small D.H. and Foa L.

Menzies Research Institute, University of Tasmania, Hobart, 7001, Tasmania, Australia.

Calcium is a ubiquitous second messenger within cells, and is a crucial mediator of growth cone motility. Store-operated calcium entry (SOCE) is a necessary process for the repletion of intracellular calcium stores, after depletion. STIM1, a calcium sensing protein, is located on the endoplasmic reticulum membrane, and signals to Orai proteins. Orai proteins are the main components of calcium release activated channels (CRAC), on the plasma membrane, and STIM1 is known to regulate CRAC channel formation after calcium store depletion. The hypothesis of this study is that STIM1 and Orai1/2 are present within embryonic rat dorsal root ganglionic (DRG) neurons, and that STIM1 is necessary for the regulation of SOCE and hence growth cone turning. This was examined with the use of an in vitro growth cone turning assay, morpholino knockdown, immunofluorescence analyses and western blot analyses. STIM1 was found to be present within DRG growth cones. STIM1 knockdown induced a switch in growth cone turning responses, from a chemoattractive response to BDNF ( $10.64 \pm 1.62^\circ$ ; n=24) into a chemorepulsive response ( $-7.02 \pm 2.64^\circ$ ; n=16; p<0.0001). A normal chemorepulsive response to semaphorin 3A ( $-7.90 \pm 2.94^\circ$ ; n=16) was abolished by STIM1 knockdown ( $0.95 \pm 2.48^\circ$ ; n=19; p<0.05). STIM1 and Orai1/2 staining suggested that different staining patterns, diffuse and punctate, occur within growth cones depending upon the calcium store status, with areas of probable co-localisation. This data suggests that the two proteins work together to regulate SOCE in growth cones. These data provide evidence that SOCE, mediated by STIM1 and Orai is necessary for growth cone motility and accurate axon pathfinding. This work has significant implications for development and nerve regeneration.



## POS-TUE-013

**AXON GUIDANCE BY GROWTH RATE MODULATION DOMINATES OVER BIASED TURNING IN SHALLOW GRADIENTS**

Pujic Z.<sup>1</sup>, Mortimer D.<sup>1</sup>, Vaughan T.<sup>1</sup>, Thompson A.W.<sup>1</sup>, Feldner J.<sup>1</sup>, Vetter I.<sup>1</sup> and Goodhill G.J.<sup>1,2</sup>

<sup>1</sup>Queensland Brain Institute. <sup>2</sup>School of Mathematics and Physics, The University of Queensland, St Lucia, QLD 4072, Australia.

Guidance of axons by molecular gradients is crucial for wiring up the developing nervous system. It is often assumed that the unique signature of such guidance is immediate and biased turning of the axon tip towards or away from the gradient. However, here we show that such turning is not required for guidance. First, we analyzed neurite growth from dorsal root ganglion (DRG) explants grown in precisely controlled shallow gradients of nerve growth factor (NGF). Although there was more growth up the gradient than down the gradient (Mortimer et al, PNAS, 106:10296-301, 2009), there was little neurite turning ( $n \sim 66$  explants per condition). Second, we showed that the lack of observable turning was not due to trophism, by showing that neurites directed up the gradient grew more than neurites directed down the gradient, even when the former were at a lower absolute NGF concentration than the latter ( $n=93-95$  explants per condition). Third, we showed that the lack of observable turning was not due to turning having already occurred within the explant ( $n = 60-120$  explants per condition), or to subsequent straightening of initially curved trajectories ( $n = 6$  trajectories). Fourth, we showed that a computational model based on modulating the speed of neurite growth depending on gradient direction fit our data, whereas a computational model based on biased turning did not ( $n \sim 66$  explants per condition). Lastly, we showed that such growth-rate modulation does not occur in the steep gradients of the pipette assay. Thus, biased turning dominates in steep gradients while growth-rate modulation dominates in shallow gradients. These results reveal a previously unidentified mechanism for the directed development of neural connections.

## POS-TUE-015

**CHANGES IN LYSOSOMAL-LIKE ACTIVITY DURING DEVELOPMENT OF HEARING IN RAT AUDITORY BRAINSTEM**

Xian S., Sen M. and Rodriguez-Contreras A.  
Biology Department, CCNY, CUNY, New York, NY 10031.

The onset of hearing in rodents occurs during a sensitive period in postnatal development. In the auditory brainstem, the sensitive period is characterized by changes in the pattern of electrical activity and an increased expression of extracellular matrix components (perineuronal nets). Previous studies also demonstrated pruning of the inhibitory synaptic connection between the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO), which form part of a brainstem circuit involved in sound localization. Previous data from our lab indicate that MNTB afferents undergo structural pruning during postnatal development (Rodriguez-Contreras et al., 2008), suggesting that synapse elimination is another correlate of the sensitive period. What are the cellular mechanisms that underlie synapse elimination in auditory circuits? To begin to address this question we performed vital staining experiments in brainstem slices of P09-17 rats ( $n=17$ ). We used a lysosome-specific fluorescent probe (LysoTracker Red) to explore a link between phagocytic activity and synaptic pruning in the MNTB. We observed an increase in the density of LysoTracker-stained particles as a function of age ( $P09-11 = 4.67 \pm 1.26$ ;  $P12-14 = 6.82 \pm 0.96$ ;  $P15-17 = 1.68 \pm 0.21$ ). Lysosome-like labeling was also observed in the region of the LSO at P12, suggesting a link between synapse elimination and increased phagocytic activity. Using a combination of LysoTracker staining, neural tracing and immunohistochemistry allowed us to track LysoTracker-stained particles to neuropil regions and to S100 $\beta$  cells, which we presume are glial cells. These results suggest that axonal refinement in the auditory brainstem is driven by autophagic processes similar to those recently described at the neuromuscular junction (Song et al., 2008).

## POS-TUE-014

**A UNIFYING MODEL OF RETINOTECTAL MAP FORMATION REVEALS A CRITICAL ROLE FOR AXON-AXON INTERACTIONS**

Simpson H.D.<sup>1</sup> and Goodhill G.J.<sup>1,2</sup>

<sup>1</sup>Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia. <sup>2</sup>School of Mathematics and Physics, The University of Queensland, St Lucia, QLD 4072, Australia.

Retinal axons form a topographic map in the optic tectum / superior colliculus during development. Data from both surgical and genetic manipulations across a range of species show that chemospecificity and competition play essential roles in the correct formation of the map. Here we present a simple but realistic computational model of retinotectal / retinocollicular map formation, based on multiple activity-independent influences, that provides a unifying explanation for the results of both surgical and genetic manipulations. First, we show that the model is consistent with several typical features of normal development, such as realistic ingrowth and axonal trajectory shape. Second, we show that surgical or 'systems level' manipulations may be explained in the model using only a chemoaffinity rule combined with competition, but that the model is also able to reproduce the retinotopy seen in sparse conditions where there is little or no competition between axons. Thirdly, we demonstrate that to explain the map duplication and collapse observed in *Is/2* EphA knock-in experiments it is necessary to add axon-axon interactions based on receptor ratio evaluations. Overall the model shows that simple mechanistic rules are sufficient to unify a wide range of apparently disparate data in retinotectal map formation, including different classes of experiment in different model species.

## POS-TUE-016

**REGULATION OF MOTONEURON SURVIVAL AND INNERVATION DURING EMBRYONIC DEVELOPMENT**

Fogarty M.J.<sup>1</sup>, Yanagawa Y.<sup>3</sup>, Obata K.<sup>4</sup>, Bellingham M.C.<sup>1</sup> and Noakes P.G.<sup>1,2</sup>

<sup>1</sup>School of Biomedical Sciences. <sup>2</sup>Queensland Brain Institute, University of Queensland, QLD, 4072, Australia. <sup>3</sup>Gumma University Graduate School of Medicine, Japan. <sup>4</sup>RIKEN Brain Science Institute, Japan.

**Purpose:** Morphological and functional evidence shows that mice lacking either GABAergic (GAD67<sup>-/-</sup>) or glycinergic (Gephyrin<sup>-/-</sup>, Banks et al 2005) transmission do alter motoneuron survival in a region specific manner across during embryonic development. Here we provide evidence that the combined loss of GABAergic and Glycinergic synaptic transmission (VGAT<sup>-/-</sup> mice) produces exaggerated aberrance in motoneuron survival, muscle innervation and neuromuscular synaptic number, when compared to mice missing glycinergic and mice missing GABAergic transmission. **Methods:** Embryonic VGAT<sup>-/-</sup> and wild-type<sup>+/+</sup> mice at E13, E15 and E18 had their spinal cords and skeletal muscle (diaphragm, latissimus dorsi and gluteus maximus) dissected out and processed for histology and whole-mount immunohistochemistry respectively. **Results:** At E15 and 18 respiratory spinal motoneurons (XIIIn) was shown to have decreased survival compared to wild type ( $n=3$ ) and decreased innervation of the diaphragm at these ages ( $n=4$ ). Motoneuron count and bifurcation number was 1014 and 3.1 for mutant and 1278 and 3.8 for wild-type E18. By contrast, we observed increase numbers of lumbar motoneuron and hind limb innervation in VGAT<sup>-/-</sup> mice at E15 and E18 ( $n=4-6$ ). Motoneuron count and bifurcation number was 4146 and 5.9 for mutant and 1744 and 4.4 for wild-type E18. **Conclusion:** The results of this study indicate that there are additive effects of the loss of GABAergic and glycinergic on the regulation of motoneuron numbers and their innervation of their target muscles, but that these effects are exerted in a regional dependent manner.

## POS-TUE-017

EXPRESSION OF COMPLEMENT FACTORS IN THE SOD1<sup>G93A</sup> MOUSE MODEL OF MOTOR NEURON DISEASELee J.D.<sup>1</sup>, Woodruff T.M.<sup>1</sup>, Taylor S.M.<sup>1</sup> and Noakes P.G.<sup>1,2</sup><sup>1</sup>School of Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia. <sup>2</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072, Australia.

**Purpose:** The complement system has recently been implicated in pathogenesis of motoneuron disease (MND). Our previous studies in SOD1<sup>G93A</sup> rat model of MND demonstrated a role for the complement factors in motoneuron death. The current study aimed to determine the expression and localization of the receptors for C5a (CD88 and C5L2) and other complement components (C1qB, C3, C5, C9, C3aR and CD55) at mRNA and protein level, in SOD1<sup>G93A</sup> mouse model of MND. **Methods:** C57BL/6J SOD1<sup>G93A</sup> and wild-type (WT) mice were examined at 3 different post-natal (P) ages: P30 (pre-symptomatic); P70 (onset); and P160 (end stage) of MND. At each age, mRNA expression levels of various complement factors were investigated by RT-PCR or qPCR (n=3/age). Protein levels of CD88 and C5L2 were also determined using immuno-blotting. Localization of CD88 and C5L2 mRNA and protein within the spinal cord was determined by in-situ hybridisation and immuno-histochemistry (n=3/age). **Results:** CD88 mRNA and protein levels were increased at P160 in SOD1<sup>G93A</sup> mice compared to WT littermates. CD88 mRNA and protein were expressed on motoneurons and proliferating microglia throughout disease progression, whereas CD88 was present only on motoneurons in WT mice at all ages. By contrast, C5L2 protein expression was significantly higher in SOD1<sup>G93A</sup> mice when compared to WT mice only at P30. C5L2 mRNA and protein was localized to motoneurons in both WT and SOD1<sup>G93A</sup> mice at all ages. **Conclusion:** These results indicate that the expression of complement factors including C5a and its receptors, is increased in SOD1<sup>G93A</sup> mice, and may therefore play a role in the progression of MND in the SOD1<sup>G93A</sup> mouse.

## POS-TUE-018

## PROTEOMIC ANALYSIS OF SPINAL CORD IN RESPONSE TO INJURY AT DIFFERENT DEVELOPMENT STAGES IN MONODELPHIS DOMESTICA

Noor N.M.<sup>1</sup>, Steer D.L.<sup>2</sup>, Ek C.J.<sup>1</sup>, Richardson S.J.<sup>3</sup>, Dziegielewska K.M.<sup>1</sup> and Saunders N.R.<sup>1</sup><sup>1</sup>Department of Pharmacology, Melbourne University. <sup>2</sup>Department of Biochemistry & Molecular Biology, Monash. <sup>3</sup>School Medical Sciences, RMIT.

Spinal cord (SC) injury affects sensory and motor functions, the degree of which depends on the site of trauma. The immature SC has been shown to functionally recover from injury but mature SC does not (1). The marsupial, *Monodelphis domestica* has the ability to recover functionally and morphologically from SC injury but only if the injury occurs in the first two weeks of life (2). Newborn pups were subjected to complete spinal transection under isoflurane anesthesia at 7 or 28 days of age. Cords were removed 1 and 7 days after operation and proteomic analyses performed for the segment caudal to the injury site. Extracted proteins were fractionated based on isoelectric point and separated to subunit molecular weights by SDS PAGE. Gels were analyzed by gel analysis software, Genetools (Syngene). Results from injured animals were compared to age matched controls. Mass spectrometry was performed on P7+1days (P8 control) and P28+7days cords (P35 control). Several differentially expressed proteins were identified. These belong to: cytoskeleton networks; signaling, apoptotic or protein degradation pathways or are involved in regulation and stress response, neurite growth and protein synthesis. 14-3-3 proteins, cofilin and ubiquitin, implicated in apoptosis regulation and axonal growth guidance, showed age related differential expression. Current efforts include cellular localization of identified proteins and further analysis as more results become available. (1) Fry, E. & Saunders, N. (2000). Clin Exp Pharm Physio, 27, 542-547. (2) Fry, E., Stolp, H., Lane, M., Dziegielewska, K. & Saunders, N. (2003). J Comp Neurol, 466, 422-444.

## POS-TUE-019

## DIFFERENTIALLY EXPRESSED GENES IN THE INJURED SPINAL CORD OF EPHA4 KNOCKOUT MICE

Munro K.M., Perreau V.M. and Turnley A.M.  
Centre for Neuroscience, The University of Melbourne, Australia.

Mice lacking the developmental axon guidance molecule EphA4 show extensive axonal regeneration and functional recovery following spinal cord injury. Alterations in the level of astrocytic gliosis and the vascular response to injury have previously been identified in EphA4 knockout mice. In this study we have examined differentially expressed genes which may be important for the axonal regeneration observed. **Methods:** Adult EphA4 knockout and wild-type mice given a lumbar spinal cord hemisection were compared to those with sham injury (laminectomy only). RNA from injury epicentre taken at 4 days post-injury or equivalent tissue in control mice (n=3 per group) was individually hybridised to Affymetrix Mouse All-Exon Array 1.0 chips. Microarray data was analysed with Partek Genomics Suite to identify differentially expressed genes. Histological examination of genes of interest was conducted at 4 days post-injury (n=3 per group). **Results:** A two-way ANOVA identified 90 genes differentially expressed in response to injury in EphA4 knockout compared to wild-type mice (p<0.01). These included inflammatory phospholipid-related genes LPA1 (p=0.002), a receptor for lysophosphatidic acid, and alkaline ceramidase 2 (ACER2, p=0.006), a regulator of sphingosine production. By histological examination, at 4 days post-injury LPA1 was localised to reactive astrocytes and ACER2 was localised to a subset of reactive astrocytes, microglia and macrophages. RT-PCR indicated LPA1 and ACER2 gene expression in postnatal astrocyte cultures, suggesting lysophospholipid responsiveness may play a role in the altered astrocytic gliosis observed in EphA4 knockout mice.

## POS-TUE-020

## LONG TERM REDUCTIONS IN GLUTAMATERGIC NMDA AND DOPAMINERGIC D1 AND D2 RECEPTORS IN THE RAT BRAIN FOLLOWING ADOLESCENT, BUT NOT PERINATAL, MK-801 TREATMENT

Newell K.A.<sup>1,2</sup>, Dawson A.E.<sup>1,2</sup> and Huang X.F.<sup>1,2</sup><sup>1</sup>Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia.<sup>2</sup>Schizophrenia Research Institute, 384 Victoria St, Darlinghurst, 2010, NSW, Australia.

The glutamatergic NMDA receptor plays a key role in perinatal and adolescent brain development. Hypofunction of NMDA receptors, especially during key periods of brain development, is hypothesized to play a role in schizophrenia aetiology and pathophysiology. The aim of this study was to investigate the long-term effects of perinatal and adolescent NMDA receptor antagonism on glutamatergic and dopaminergic receptors in the rat brain. **Methods:** Sprague-Dawley rats were treated with the highly specific NMDA receptor antagonist MK-801 (0.3-0.5mg/kg) or saline on postnatal days 7, 9, and 11 (the perinatal period) or on postnatal days 42, 44, and 46 (the adolescent period). Rats were sacrificed at 12 weeks of age (adulthood) and brains collected (n=5/group). Receptor autoradiography was used to measure glutamate NMDA receptor and dopamine D1 and D2 receptor levels in the prefrontal cortex, hippocampus and striatum. **Results:** NMDA receptor binding density was significantly reduced in the prefrontal cortex (13.1%; p<0.05) and hippocampus (14.4%; p<0.05) of adolescent MK-801 treated rats compared to saline controls, but was unchanged following perinatal MK-801 treatment. D1 receptor binding density showed small (<10%) but significant (p<0.05) reductions in the prefrontal cortex and striatum of adolescent MK-801 treated rats compared to controls, while D2 receptor binding density was also reduced in the striatum of adolescent treated rats (p<0.001). D1 and D2 receptor binding density was unchanged following perinatal MK-801 treatment. **Conclusion:** These results show that adolescent, but not perinatal, NMDA receptor antagonism induces long-lasting changes in NMDA, D1 and D2 receptors in the rat brain. This suggests differing sensitivities of the perinatal and adolescent brains to NMDA receptor antagonism and highlights a vulnerability of the adolescent brain to NMDA receptor hypofunction.

## POS-TUE-021

## BINGE DRINKING AND THE ADOLESCENT BRAIN

Rae C.<sup>1</sup>, Mewton L.<sup>2</sup>, Henry R.G.<sup>3</sup> and Teesson M.<sup>2</sup><sup>1</sup>Prince of Wales Medical Research Institute and UNSW. <sup>2</sup>National Drug and Alcohol Research Centre, UNSW. <sup>3</sup>Dept of Radiology, UCSF.

Adolescence is a critical period for brain development, with active rewiring of circuitry necessary in successful development of adult adaptive patterns of behaviour. Binge drinking amongst adolescents is of deep concern considering the capacity for interference with the development of these important circuits. The available evidence suggests that heavy adolescent alcohol consumption disrupts cortical development in a manner that promotes continued impulsive behaviour, alcohol abuse and risk of alcohol dependence. However, there are few studies of the brain targeted to binge drinking effects in adolescent humans. We obtained binge-drinking histories, cognitive data (STROOP, emotional face recognition, depression and anxiety measures) and magnetic resonance imaging (structural, diffusion tensor and spectroscopy) on a group of adolescents ( $N \geq 20$ ; males and females aged 16-17 years). This group included non-drinking adolescents (controls). Binge drinkers show significant impairments on the STROOP task with slower responses and greater errors ( $P < 0.03$ ), poorer emotional recognition (FACES: angry/afraid  $P < 0.05$ ) compared to non-drinkers. These neuro-cognitive changes were positively correlated with binge drinking episodes and alcohol consumption. Drinkers had significantly elevated dorsolateral prefrontal cortex glutamate (Glu) levels compared to non-drinkers, and these correlated with depression and anxiety scores ( $r^2 = 0.7$ , ( $P = 0.05$ ) and  $0.91$  ( $P = 0.001$ )) respectively. Our data indicated a non-linear response of the DLPFC to alcohol, with increased Glu relative to alcohol consumption in males (amount of binge/length of time since first binge) until a point is reached when there are signs indicative of brain damage. An extreme binger (17 drinks per session twice per week) had NMR spectra indicative of damage, with significantly less ( $> 2$  SD) NAA, Cre, MI and Glu, and more choline than the average for males as well as visible lactate, a compound rarely seen in normal adolescent brain.

## POS-TUE-023

## CONCENTRATION OF CORTISOL IN HUMAN HAIR UNDER REST AND PAIN CONDITIONS

Sharples C.F., Kauter K. and McFarlane J.  
University of New England, Armidale, NSW.

Four investigations were conducted into the concentration and responsivity of cortisol in adult human hair. Concentration across body sites ( $n=10$  males) varied significantly, with highest values in the arms, followed by legs, with the scalp being lowest. However, concentrations within-shaft from a single site were significantly correlated in longer female ( $n = 12$ ) hair. Two studies of concentration changes following 1 min immersion in ice water (00C to 40C) were also conducted. The first study ( $n = 3$  males) showed immediate, brief and localized increases in cortisol from hair on the immersed forearm but not from hair on the opposite lower leg. The second study ( $n = 5$  males) showed further localization of hair cortisol changes along the forearm, with independent responses being observed in areas only 250mm apart. These results are considered within a model of localized anti-inflammatory hair cortisol responses to trauma and add to our knowledge of the peripheral cortisol synthesis system in human hair.

## POS-TUE-022

FUNCTIONAL RECOVERY AND MORPHOLOGICAL REPAIR FOLLOWING SPINAL CORD INJURY IN THE DEVELOPING OPOSSUM (*MONODELPHIS DOMESTICA*)Wheaton B.J., Callaway J.K., Ek C.J., Dziegielewska K.M. and Saunders N.R.  
Dept. Pharmacology, University of Melbourne.

Complete spinal cord injury (SCI) usually results in permanent loss of motor and sensory function below the lesion. Previous work in neonatal opossums has shown that, following SCI, projection of axons through the injury site and functional recovery are possible, but these abilities decline with age (Fry et al., J. Comp. Neurol., 466, 422-444, 2003). A key question is to what extent functional recovery is possible in older animals and how this relates to axonal growth. METHODS: The spinal cords of opossum pups at 7- or 28-days of age ( $n=6$  per group), attached to the anaesthetised mother, were completely transected in mid-thoracic region under sterile conditions. Three months post-injury the animals' locomotion was assessed using random rung ladder, BBB and swimming tests. Following these tests bilateral injections of Fluororuby were made below the lesion to label descending axons. RESULTS: Opossums injured at P28 scored significantly lower on the BBB scale than the P7-injured ( $12 \pm 0.3$  vs  $17 \pm 1.4$ ) and control animals ( $21 \pm 0$ ). In the ladder test P28-injured animals made more footslips than P7-injured animals and in the swimming test P7-injured animals regained some ability to use their hindlimbs but P28-injured animals did not. Morphological examination of P7-injured cords revealed the formation of a tissue bridge across the lesion site. This bridge was absent in P28-injured animals. Backlabelled neurons were abundant in brainstems of control and P7-injured animals, but were nearly absent in P28-injured animals. DISCUSSION: In this study both P7- and P28- injured opossums could walk with hindlimb-forelimb co-ordination, but only P7-injured animals could swim using their hindlimbs. The swimming test and axon tracing results indicate that the locomotor function of P28-injured animals did not involve supraspinal input to regions below the lesion and presumably involved changes in local spinal circuits. Supported by the Victorian Neurotrauma Initiative (Project DPO48).

## POS-TUE-024

TEMPORAL PATTERNS OF BONE FORMATION DURING FETAL GROWTH IN *PTEROPUS POLIOCEPHALUS*Gear R.B. and O'Brien G.M.  
Human Biology and Physiology, UNE, NSW 2351.

Histology is being used to analyse the transitions from a cartilage model, to osteoid, then to calcified bone matrix during skeletal development. The aim is to determine the timecourse of bone development in the fetus, and eventually design a staging system for fetal development, comparable to the Carnegie stages for embryo development. ImageJ was used to measure Goldners or alcian blue stained wax sections of decalcified bone. The proximal epiphysis and mid-diaphysis were examined in the humerus, radius, femur, and tibia from 17 *Pteropus poliocephalus*, greyheaded flying-fox, fetuses that had been sourced from stored collections. A hyaline cartilage model of the mid-diaphysis of the tibia was present in the very early fetal specimens, but had already been replaced by osteoid in the humerus, radius and femur; the tibia was the last of the four bones to develop. Osteoid and some calcified bone matrix was present in the humerus, radius, femur and proximal epiphysis of the tibia for all stages of development monitored. Total cross-sectional areas of the epiphyses grew faster in forelimbs than hindlimbs ( $P < 0.01$  Students T-test) as did bone cavities during subsequent remodelling ( $P < 0.01$  Students T-test). In the diaphysis, growth of the humerus proceeded fastest and that of the tibia was slowest ( $P < 0.01$  Students T-test), but there was no difference between bones in the rate of remodelling of the diaphyses. The typical antero-posterior sequence of development was confirmed. A cartilage model provided a scaffold for osteoid, which was replaced by calcified bone: all three tissues were present early in fetal life. These results provide the necessary information for interpretation of computed tomography images of dense regions and cavities that are being used to assess skeletal development.



## POS-TUE-025

## CHARACTERISATION OF THE AROMATASE-EGFP TRANSGENIC MOUSE

Chua H.K.<sup>1</sup>, Horne M.<sup>1,2</sup> and Boon W.C.<sup>1,2,3</sup><sup>1</sup>Florey Neuroscience Institutes, Parkville, VIC 3052, Australia.<sup>2</sup>Centre for Neuroscience, The University of Melbourne, Parkville, VIC 3010, Australia. <sup>3</sup>Dept Anatomy and Developmental Biology, Monash University, Clayton, VIC 3800, Australia.

Aromatase (Cyp19a1) is a cytochrome P450 enzyme that converts androgens to estrogens. It is expressed mainly in the granulosa cells of the ovary but is also expressed in other tissues such as testis and brain. This suggests that estrogens can be produced by various tissues in male and female animals. Due to the high structural similarity the protein shares with other members of the cytochrome P450 superfamily, and the lack of a readily available and specific antibody, our study of aromatase protein expression was carried out using the Enhanced Green Fluorescence Protein (EGFP) tagged aromatase transgenic mouse model from the Mutant Mouse Regional Resource Centers, USA. This transgenic mouse carries a mouse genomic bacterial artificial chromosome which contains the coding sequence for EGFP, followed by a polyadenylation signal which is inserted at the ATG translation initiation codon of the Cyp19a1 gene. The expression of the reporter mRNA/protein is thus driven by the promoters of the mouse Cyp19a1 gene. We have reported previously that the promoter regions of the Cyp19a1 gene is fairly complex with tissue-specific promoters and untranslated first exons. In this study, we have characterised the Aromatase-EGFP mouse, and showed both male and female transgenic mice have normal gross anatomy and reproduced normally with the Mendelian distribution of the transgene. Similar to previous reports, we have observed EGFP in tissues that expressed aromatase, such as the granulosa cells in the ovaries. We have also detected the expression of EGFP by immunostaining in brain regions (eg hypothalamus) which have been previously demonstrated by in situ-hybridisation to express aromatase. In addition, EGFP immunostaining in the cortex of both male and female adult brains were also observed. In summary, the EGFP expression is driven by the Cyp19a1 regulatory sequences to express specifically in the ovary, testis and brain of the Aromatase-EGFP mice.

## POS-TUE-026

## INTERSTITIAL CELLS OF CAJAL IN THE MOUSE REPRODUCTIVE TRACT

Gravina F.S., Jobling P., de Oliveira R.B., Kerr K.P. and Van Helden D. School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW 2308.

The pacemaker mechanism activating spontaneous contractions in the uterus and hence the expulsion of the foetus remains poorly understood. A recent finding that could advance understanding of this has been the discovery that cells resembling pacemaker cells in the gastrointestinal tract termed Interstitial Cells of Cajal (ICCs) are also present in the uterus. However, it is not yet clear whether these cells play a role in uterine pacemaking. The present research addresses this issue by comparing the presence and functional properties of ICCs-like in the uterus to that in the cervix and vagina of non-pregnant mice. Female Swiss mice (6-10 weeks) were euthanased by overexposure to the inhalation anaesthetic isoflurane (5-10%), a procedure approved by the Animal Care and Ethics Committee at the University of Newcastle. ICCs-like and smooth muscle cells were labelled by fluorescence immunohistochemistry using a rat anti-CD117 antibody visualised with donkey anti-rat FITC and an alpha-smooth muscle actin conjugated with Cy3, respectively. Contractions were measured from tissues mounted under 0.5g tension in baths containing physiological solution at 37°C. The uterus, which always exhibited spontaneous contractions, contained a layer of ICCs-like and the well-reported relatively thick muscle layers (n=7). The cervix was only spontaneously active in approximately 40% of tissues (n=5); it did not exhibit a significant amount of ICCs-like and had a relatively low density of smooth muscle cells. Vaginal tissue, while exhibiting ICCs-like, was not spontaneously active and had only a thin bundle of smooth muscle (n=5). These results indicate that the presence of ICCs-like might not necessarily correlate with spontaneous activity but cell density may be a factor here.

## POS-TUE-027

## MOLECULAR DELIVERY TO THE BRAIN USING ENDOGENOUS PROTEIN TRANSPORT

Liddelow S.A., Dziegielewska K.M., Noor N. and Saunders N.R. Department of Pharmacology, the University of Melbourne.

The highly vascularised choroid plexuses within the ventricles of the brain are comprised of epithelial cells connected by tight junctions. The tissue is involved in cerebrospinal fluid (CSF) production and secretion, as well as transfer of molecules between the blood and CSF. During early development, when the brain is poorly vascularised, the choroid plexus is the main route of entry for molecules from blood into CSF. This route of entry has been shown to be transcellular and localised in a specific population of plexus epithelial cells (Liddelow et al., 2009). The present study investigated the effect changes in protein content of blood would have on the plexus protein transferring cells, together with resulting modification in CSF composition. This was achieved by increasing levels of circulating plasma protein at different stages of development and by introducing an exogenous foetal protein, fetuin. Three ages of *Monodelphis domestica* (opossum) pups were used in this study: P9 (early stage of brain and choroid plexus development), P65 (juvenile) and P110 (adult), n=6 at each age and time point. Animals were injected intraperitoneally with either adult *Monodelphis* plasma (250µg protein/g body weight) or bovine fetuin (CalBiochem, 250µg/g body weight). At the end of the experimental period the animals were terminally anaesthetised (inhaled isoflurane) and CSF, blood and brains collected. Brains processed for histology. Results show that introducing an inappropriate protein into the circulation lead to changes in the transfer of proteins across the blood-CSF barrier, reflected in the changed protein composition of the CSF and the number of protein transferring cells of the choroid plexus. In addition, once in the CSF, fetuin was readily taken up by some cortical neurons.

## POS-TUE-028

## EXPRESSION AND FUNCTION OF GHRELIN AND RECEPTORS IN HUMAN ENDOMETRIAL CANCER CELL LINES

Fung J.N.T. and Chen C. School of Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia.

**Purpose:** Endometrial cancer is the most common malignant tumour in female reproductive tract. This study has examined the expression and function of two new facets of the growth hormone axis, the growth hormone secretagogue receptor (GHS-R) and its endogenous ligand ghrelin, in endometrial cancer cells. **Methods:** Four human endometrial cancer cell lines with different differentiation were used (Ishikawa, HEC1A, HEC1B and KLE). Ghrelin and its receptors GHS-R 1a and GHS-R 1b mRNA expression was detected by RT-PCR and quantified with qPCR by normalising to 18s rRNA. The protein expression of GHS-R1a was also detected by immuno-blotting with specific antibodies. Effect of ghrelin on endometrial cancer cell proliferation was determined by using the MTS dye method. Endometrial cancer cell lines were cultured in presence or absence of human n-octanoylated ghrelin at concentrations ranged from 0.1 to 1,000 nM for 24, 48 or 72 hours (n=3/ time point). **Results:** Ghrelin, GHS-R1a and GHS-R1b gene expression was detected in all cell lines. Quantification of mRNA level demonstrated that both receptor isoforms gene expression is highly and positively associated with the differentiation level of the cell lines. GHS-R1b gene expression of poorly differentiated KLE endometrial cancer cell line is 3.9-fold higher than that of well differentiated endometrial cancer cell line Ishikawa. Protein expression of GHS-R1a was detected in all four endometrial cancer cell lines. Ghrelin treatment significantly increase the cell proliferation of Ishikawa, HEC1B and KLE cell lines by 32%, 29% and 28% above untreated controls after 72 hours incubation. **Conclusion:** This study demonstrates the expression of the GHS-R and ghrelin in endometrial cancer cell lines and circulating ghrelin may promote cancer cell proliferation.

## POS-TUE-029

**GHRELIN AND APPETITE REGULATION IN THE SPINIFEX HOPPING MOUSE**

McLeod J.L., Trajanovska S., Chung S. and Donald J.A.  
Deakin University, Geelong, Victoria, Australia 3217.

Ghrelin is a hormone released from the gut that stimulates food intake, and is important in the control of energy balance. The water-deprived Spinifex hopping mouse, *Notomys alexis*, exhibits a natural cycle of fasting, followed by sustained food intake that is greater than animals with access to water. Food intake is increased to generate metabolic water in order to maintain fluid homeostasis during water deprivation (WD). We hypothesised that an important driver of the increased appetite is ghrelin. Five groups (n = 5) of hopping mice were subjected to WD with unlimited food availability over a time course of 29 days. Food intake and body weight were determined each day and compared to a control group with access to water. Plasma and tissue samples were collected at five time points (days 0, 2, 5, 10 and 29), and the level of plasma ghrelin and brain ghrelin receptor (GHSR1a) mRNA were determined by ELISA and real time-PCR, respectively. Plasma ghrelin concentration mirrored the decreased food intake during the first five days of WD, rising significantly above control (Day 0) at day 10 ( $p < 0.05$ ) and then decreasing markedly in the second phase of WD. Brain GHSR1a mRNA expression peaked at day 2 of WD when plasma ghrelin levels were lowest, then decreased to control levels for the remainder of the experiment. The data suggest ghrelin is important in stimulating the increase in food intake in hopping mice during the first phase of WD. However, ghrelin signalling appears to be down-regulated in the sustained appetite drive of the second phase of WD, suggesting that other signalling systems are involved in appetite regulation during this phase.

## POS-TUE-030

**A FUNCTIONAL ROLE FOR CANNABINOID RECEPTORS IN THE KIDNEY PROXIMAL TUBULES**

Jenkin K.A., Grinfeld E., McAinch A.J. and Hryciw D.H.  
School of Biomedical and Health Sciences, Victoria University.

The current obesity epidemic has led to an increased rate of many obesity related comorbidities such as Type 2 diabetes. This places considerable pressure on the health care system with increased rates of diabetes and obesity leading to numerous medical complications such as end stage renal failure. Endocannabinoids bind to a small number of identified receptors including Cannabinoid receptor 1 (CB1) and Cannabinoid receptor 2 (CB2), Transient receptor potential cation channel subfamily V member 1 (TRPV1) and the putative cannabinoid receptor, GPR55. The cannabinoid receptors although initially thought to be predominantly located in the brain and central nervous system, are located in a number of peripheral tissues. Importantly, despite their abundance in kidneys, the functional role of the endocannabinoid system in the renal system has largely been overlooked. This study aims to establish which cannabinoid receptors are expressed in rat kidney tissue and specifically in the proximal tubule via the cell line Human Kidney-2 (HK2), as well as the functional role of the cannabinoid receptors in HK2 cells. Using RT-PCR analysis of mRNA extracted from rat kidney and HK2 cells, it was found that all four cannabinoid receptors CB1, CB2, TRPV1 and GPR55 were expressed in these samples. Further, western blot analysis of protein samples determined that all receptors were expressed in kidney and proximal tubule samples. In addition, using HK2 and assessing cell viability using the MTT assay, it was found that the cannabinoid receptors significantly influenced cell viability in proximal tubule cells. In summary, we have characterised expression of cannabinoid receptors in kidney tissue and proximal tubule cells which will improve our understanding of how cannabinoid receptors influence normal kidney function.

## POS-TUE-031

**ZUCKER OBESE (FA/FA) RATS, COMPARED TO SPRAGUE-DAWLEY RATS, HAVE FEWER EPISODES OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS**

Kontos A.<sup>1</sup>, Ootsuka Y.<sup>1,2</sup>, de Menezes R.<sup>1,3</sup> and Blessing W.<sup>1</sup>  
<sup>1</sup>Centre for Neuroscience, Dept. of Human Physiology, Flinders University, Adelaide, Australia. <sup>2</sup>Dept. of Physiology, Kagoshima University, Kagoshima, Japan. <sup>3</sup>NUPEB, Federal University of Ouro Preto, Ouro Preto, Brazil

**Purpose:** In Sprague-Dawley rats, brown adipose tissue (BAT) temperature suddenly increases in an episodic burst-like fashion approximately every 80-100 min during the dark active phase of the circadian cycle. This ultradian rhythm contributes to a highly correlated increase in body and brain temperature that occurs during periods of behavioral activity (1). Obese fa/fa Zucker rats (no functioning leptin receptors) have reduced behavioral activity (2). We investigated the dark-active phase ultradian rhythms in BAT, brain and body temperatures, and their correspondence with behavioral activity, in Zucker rats (n=10, aged 11-18 weeks, weight 435-660 g). **Methods:** Temperature variables were measured and analyzed as previously described (1). Behavioral activity was measured electronically using a grid of laser light beams. **Results:** During the dark active period increases in BAT temperature, occurring together with highly correlated increases in brain and body temperature and behavioral activity, occurred every  $124 \pm 5$  min (mean  $\pm$  SEM), significantly greater ( $P < 0.001$ ) than  $86 \pm 3$  min, the corresponding values for Sprague Dawley rats in the same experimental conditions (data from ref 1). Amplitude of the BAT temperature increases in Zucker rats ( $1.22 \pm 0.04^\circ\text{C}$ ) was significantly greater ( $P < 0.05$ ) than  $1.10 \pm 0.03^\circ\text{C}$ , the corresponding value in Sprague Dawley rats (data from ref 1). **Conclusion:** Our study demonstrates that obese Zucker rats have larger, less frequently occurring episodic ultradian increases in BAT, body and brain temperature, and that these increases are still coordinated with corresponding increases in behavioral activity. (1) Ootsuka et al., Neuroscience 2009. (2) Towa et al. Exp Animal, 2004.

## POS-TUE-032

**LEUCINE SUPPLEMENTATION IMPROVES GLUCOSE METABOLISM IN OFFSPRING FROM OBESE DAMS WITHOUT AFFECTING FEEDING REGULATION**

Chen H.<sup>1,2</sup> and Morris M.J.<sup>2</sup>  
<sup>1</sup>Department of Medical and Molecular Bioscience, University of Technology, Sydney, NSW 2007. <sup>2</sup>Department of Pharmacology, University of New South Wales, NSW 2052.

The hypothalamic fuel sensor mammalian target of rapamycin (mTOR) is critical for appetite regulation, through its inhibition of the potent appetite stimulator neuropeptide Y (NPY). Previous studies in our lab showed that maternal obesity caused reduced hypothalamic mTOR expression in offspring during the suckling period, accompanied by increased milk intake during suckling and greater energy intake immediately after weaning. The known mTOR agonist leucine can cross the blood brain barrier. We hypothesized that using L-leucine in drinking water to activate hypothalamic mTOR could ameliorate the hyperphagic phenotype induced by maternal obesity. **Method:** Female Sprague Dawley rats were fed chow or high-fat-diet (HFD) for 5 weeks before mating, throughout gestation and lactation. At 20 days, male pups from obese dams were weaned onto either chow or HFD diet. Within each dietary cohort, half of the pups had leucine in the drinking water. The pups from the lean dams were weaned onto the chow diet only with normal water. At 13 weeks, leucine supplementation led to a non-significant 9% reduction in both 24h energy intake and body weight in chow-fed offspring only; whereas it did not affect the weight gain of HFD-fed rats. L-leucine significantly reduced the blood glucose levels in HFD-fed rats during the entire glucose tolerance test and halved the insulin level in chow-fed rats. Hypothalamic NPY and Y2 receptor mRNA was downregulated in offspring from obese dams independent of post-weaning diet. L-leucine treatment did not affect these markers. Therefore, leucine may impact on pathways other than appetite circuitry to improve glucose metabolism.

## POS-TUE-033

**CENTRAL MECHANISMS OF LEPTIN RESISTANCE IN HYPERPHAGIC OBESE MICE WITH METABOLIC SYNDROME**

Heydet D., Larter C. and Farrell G.

Liver Research Group, ANU Medical School, Australian National University at The Canberra Hospital, Canberra, 2605.

Diet-induced obesity is associated with hyperphagia and increased serum leptin, a central satiety regulator. We studied hypothalamic changes related to leptin resistance in *Alms1* mutant (*foz/foz*) mice, a murine model of Alström syndrome, a form of monogenic obesity associated with cilia disorder, diabetes and metabolic syndrome. Serum leptin was measured in gps of *foz/foz* and wildtype (WT) mice at 3wk (weaning), 8 and 18wk of age ( $n > 5$ ), fed either rodent chow or high-fat (HF) diet. Hypothalamic proteins were estimated by western blot, mRNA expression by semi-quantitative real-time PCR. The role of neuronal primary cilia was assessed by counting number of ciliated cells. Serum leptin levels were not significantly different in weanling *foz/foz* mice compared to WT littermates. By 8wk, serum leptin increased in chow or HF-fed *foz/foz* and HF-fed WT mice, with greatest elevation in HF-fed *foz/foz* mice; levels continued to increase with time. *Foz/foz* mice exhibited decreased numbers of hypothalamic ciliated neurons. Recently, it has been proposed that neuronal cilia express leptin receptor (Ob-R). While leptin hypothalamic Ob-R protein did not change, there was a significant increase in SOCS3 at 8wk and PTP1B protein at 18wk in *foz/foz* mice; both proteins inhibit leptin receptor signaling. Induction of hyperleptinemia by HF feeding is exacerbated in this genetic model of obesity in association with decreased neuronal cilia and increased expression of molecular inhibitors of the Ob-R pathway. These data indicate that obesity in *foz/foz* mice may be driven by two different pathways of leptin resistance, one structural and the other molecular. Further studies in this model should clarify central mechanisms of leptin resistance.

## POS-TUE-034

**NOVEL ACTIN FILAMENTS REGULATE GLUCOSE CLEARANCE, INSULIN SENSITIVITY AND INSULIN SECRETION**Lucas C.A.<sup>1</sup>, Kee A.J.<sup>1</sup>, Szokolai R.C.<sup>1</sup>, Martel N.<sup>2</sup>, Laybutt R.<sup>3</sup>, Leong G.M.<sup>2</sup>, Muscat G.E.O.<sup>2</sup>, James D.E.<sup>3</sup>, Cooney G.J.<sup>3</sup> and Hardeman E.C.<sup>1</sup><sup>1</sup>Department of Anatomy, School of Medical Sciences, University of NSW. <sup>2</sup>Institute for Molecular Biosciences, University of QLD.<sup>3</sup>Diabetes and Obesity Program, Garvan Institute of Medical Research, NSW.

The onset of Type 2 diabetes is associated with alterations in both glucose uptake and insulin secretion. Glucose uptake involves a shift of Glut-4 vesicles from intracellular stores to the cell surface, whilst insulin secretion involves the fusion of insulin-containing granules with the pancreatic  $\beta$ -cell surface. We have identified novel actin cytoskeletons defined by the cytoskeletal tropomyosin (Tm) isoform Tm5NM1. Experimental analysis using Tm5NM1 transgenic (Tg) mice suggests these filaments play a role in both glucose uptake and basal insulin secretion. Tg mice have increased glucose clearance in part due to increased insulin sensitivity. The molecular events facilitating Glut4 translocation include activation of the PI3-kinase pathway and also major rearrangements of cytoskeleton components. Tm5NM1 Tg mice showed no change in insulin-stimulated Akt phosphorylation suggesting Tm5NM1 is acting downstream of insulin signalling. Using gene expression profiling with a dedicated microarray, an increase in genes involved in GLUT4 trafficking and actin filament turnover was detected in adipose tissue from Tg mice. The gene expression of genes involved in Glut-4 trafficking was examined by quantitative real-time PCR analysis ( $n=10$ /gp). Two genes *Myo1c* and *Sec8* were increased in Tg adipose tissue ( $P < 0.05$  and  $P < 0.005$  respectively). *Myo1c* and *Sec8* were also increased at the protein level (Western blot). We propose that Tm5NM1 induces more stable cortical actin filament network in adipocytes leading to the accumulation of GLUT4 trafficking machinery and enhancing glucose uptake.

## POS-TUE-035

**DEPLETION OF BRAIN NORADRENERGIC NEURONS, INCLUDING LOCUS COERULEUS NEURONS, REDUCES THE FREQUENCY OF THE EPISODIC ULTRADIAN OCCURRENCES OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS**Ootsuka Y.<sup>1,2</sup>, de Menezes R.<sup>1,3</sup> and Blessing W.<sup>1</sup><sup>1</sup>Centre for Neuroscience, Dept. of Human Physiology, Flinders University, Adelaide, Australia. <sup>2</sup>Dept. of Physiology, Kagoshima University, Kagoshima, Japan. <sup>3</sup>NUPEB, Federal University of Ouro Preto, Ouro Preto, Brazil

**Purpose:** Brown adipose tissue (BAT) temperature suddenly increases in an episodic manner, approximately every 95 min during the active phase of the circadian cycle. This ultradian rhythm contributes to a highly correlated increase in brain temperature that occurs during activity associated with brain arousal <sup>1</sup>. The locus coeruleus noradrenaline-synthesizing neurons are important regulators of arousal state <sup>2</sup>. We investigated whether lesioning these neurons with a monoclonal antibody to dopamine- $\beta$ -hydroxylase-coupled saporin toxin (anti-DBH-saporin) affects ultradian rhythms in BAT temperature. **Methods:** Either anti-DBH-saporin (5  $\mu$ g in 10  $\mu$ l, Advanced Targeting Systems) or saline-vehicle was injected into the CSF of a lateral ventricle of the brain. Two weeks after the injection, temperature variables during the dark active period were measured and analyzed as previously described <sup>1</sup>. Elimination of noradrenergic neurons in locus coeruleus and their axonal processes was assessed using DBH immunohistochemistry <sup>3</sup>. **Results:** In rats treated with anti-DBH-saporin, increases in BAT temperature occurred every  $106 \pm 10$  min (mean  $\pm$  SEM), significantly greater ( $n=6$ ,  $P < 0.05$ ) than  $85 \pm 7$  min, the corresponding value for vehicle-treated rats. Amplitude of the BAT temperature increases in the two groups was not significantly different ( $P > 0.05$ ). **Conclusion:** Our study demonstrates that after depletion of locus coeruleus neurons, rats have less frequent episodic ultradian temperature increases, suggesting that the locus coeruleus contributes to episodic ultradian BAT thermogenesis. (1) Ootsuka et al., Neuroscience 164:849, 2009. (2) Berridge, Brain Res Rev, 2008. (3) Blessing et al., Neurosci. Lett., 1998.

## POS-TUE-036

**GHS-R MRNA EXPRESSION LEVELS ARE INCREASED IN THE VMH OF DIET-RESISTANT MICE**

Yu Y.H. and Huang X.F.

Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Northfield Avenue, NSW 2522, Australia.

**Purpose:** Hypothalamic growth hormone secretagogue receptor (GHS-R) is a key receptor for ghrelin, which regulates body weight via pituitary growth hormone. Previous study reported a negative association between an upper regulated GHS-R mRNA and body fat accumulation in mice. This study examined if the GHS-R mRNA is differentially expressed between the diet-induced obese (DIO) and diet-resistant (DR) mice as well as these mice on various dietary intervention. **Methods:** Forty-five C57Bl/6 male mice were fed a high-fat diet for 8 weeks and then divided into DIO and DR mice ( $n=15$ /group), according to the highest and lowest body weight gainers, respectively. The DIO and DR mice were then randomly divided into three groups ( $n=5$ /group) and either continued on their high-fat diet (HF), changed to a low-fat diet (LF) or an energy-restricted pair-feeding diet (ER) for a further 6 weeks. **Results:** The DR mice had higher levels of GHS-R mRNA expression in the ventromedial hypothalamic nucleus (VMH) than the DIO mice on high-fat diet ( $\sim 28\%$ ,  $p < 0.05$ ). After energy-restricted pair-feeding diet for 6 weeks, the DIO mice had their body weight and fat mass reduced to normal level, which is accompanied by a significant reduction of the GHS-R mRNA expression (DIO vs DIO-ER,  $\sim 40\%$ ). Interestingly, DR-ER mice still had a significantly higher GHS-R mRNA expression than DIO-ER mice although they had the same body weight and diet following 6-week energy-restricted diet. **Conclusion:** The DR mice had a higher level of VMH GHS-R mRNA expression than the DIO mice. The body weight reduction program using energy-restricted diet is effective to lose body weight, which is accompanied by a reduction of VMH GHS-R mRNA. This study has provided evidence of central involvement in response of an elevated ghrelin after energy-restricted diet induced weight loss program.



## POS-TUE-037

**CURCUMIN REDUCES HEPATIC AND RENAL TOXICITY OF ACETAMINOPHEN IN RATS**

**Kheradpezhough E.<sup>1,2,3</sup>**, Panjeshahin M.R.<sup>1</sup>, Dehpour A.R.<sup>2</sup> and Rychkov G.<sup>3</sup>

<sup>1</sup>Shiraz University of Medical Sciences, Iran. <sup>2</sup>Tehran University of Medical Sciences, Iran. <sup>3</sup>Adelaide University, Australia.

Acetaminophen is one of the world's most popular analgesics that can be obtained over the counter. Overdose of acetaminophen, however, can cause severe damages to liver and kidneys. In this study we investigated the effects of curcumin, derived from *Curcuma Longa*, on the acetaminophen toxicity, and the possibility of combining therapy of curcumin and N-acetyl cysteine (NAC) to treat the damage caused by acetaminophen. The experiments were conducted on 72 male Sprague-Dawley rats randomly divided into 12 groups. Control group was left without treatment, and the other groups were treated with different combinations of acetaminophen, curcumin and NAC. Blood levels of AST Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea Nitrogen and Creatinine were determined 18 and 42 h after acetaminophen injection. One week later, left kidney and the caudate lobe of liver were harvested to assay Glutathione Peroxidase, Catalase and Malondialdehyde. Right kidney and the remaining lobes of liver were used for histopathology. Analysis of organ function and oxidation parameters showed that curcumin significantly reduced toxic effects of acetaminophen on liver and kidneys in a dose-dependent manner and significantly potentiated the protective effects of NAC. These findings were confirmed by histopathology. It is concluded that curcumin can protect liver and kidney from the damage caused by acetaminophen overdose. Moreover, curcumin has the potential to be used in a combination therapy with NAC, significantly decreasing the therapeutic dose of NAC and therefore its side effects.

## POS-TUE-039

**KYNURENINE PATHWAY METABOLISM IS INVOLVED IN THE MAINTENANCE OF INTRACELLULAR NAD<sup>+</sup> CONCENTRATIONS IN NEURONS AND ASTROCYTES**

**Grant R.S.<sup>1,2</sup>**, Nguyen S.<sup>1</sup> and Guillemin G.<sup>1,3</sup>

<sup>1</sup>Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney NSW. <sup>2</sup>Australasian Research Institute, Sydney Adventist Hospital, Sydney NSW. <sup>3</sup>St Vincent's Centre for Applied Medical Research, St Vincent's Hospital, Sydney NSW.

NAD<sup>+</sup> (the parent pyridine nucleotide) is involved in a number of essential cell metabolic processes from electron transport and ATP production to nuclear repair and regulation of DNA transcription. Maintenance of NAD<sup>+</sup> levels are therefore crucial to cell viability in both astrocytes and neurons with some suggestion that glia may also serve as a supplier of NAD<sup>+</sup> to neurons during times of stress. Unfortunately little detail of the pathways(s) by which these cells maintain their intracellular NAD<sup>+</sup> is available in the literature. The aim of this study was therefore to investigate the relationship, between kynurenine pathway (KP) metabolism and *de novo* NAD<sup>+</sup> synthesis in the two major cells types of the central nervous system (CNS), astrocytes and neurons. We show that inhibition of selected enzymes of the KP, results in a significant decrease in both intracellular NAD<sup>+</sup> levels and cell viability in primary human astrocytes and neurons. We also show that up-regulation of the KP by IFN- $\gamma$ , results in an increase in intracellular NAD<sup>+</sup> levels in the neuroblastoma cell line SK-N-SH, but a decrease in intracellular NAD<sup>+</sup> levels in primary astrocytes. Reduced viability and intracellular NAD<sup>+</sup> levels of primary astrocytes can be restored by supplementation with either tryptophan, a precursor of the *de novo* pathway, or via metabolism of either nicotinic acid or nicotinamide by the salvage pathway. NAD<sup>+</sup> depletion is becoming increasingly recognised as a cause of cell death in neuroinflammatory and degenerative disorders. Information from this study, showing the dependence of CNS cells on KP metabolism for NAD<sup>+</sup> synthesis, suggests the KP as a pathway of potential clinical significance.

## POS-TUE-038

**CHARACTERISATION OF THE KYNURENINE PATHWAY IN ASTROGLIOMAS**

**Adams S.<sup>1</sup>**, Braidy N.<sup>1</sup>, Grant R.<sup>1</sup> and Guillemin G.<sup>1,2</sup>

<sup>1</sup>Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia. <sup>2</sup>Department of Neuroimmunology, St Vincent's Centre for Applied Medical Research, St Vincent's Hospital, Sydney, NSW, Australia.

The kynurenine pathway (KP) is a major route of L-tryptophan catabolism leading to the production of nicotinamide adenine dinucleotide (NAD<sup>+</sup>), the anti-tumoral and neuroprotective agent, picolinic acid (PIC), and a number of other neuroactive metabolites. Evidence suggests that the induction of indoleamine 2,3- dioxygenase-1 (IDO-1) in tumour cells facilitates tumour evasion from T-cell responses. The intermediate  $\alpha$ -amino- $\beta$ -carboxymuconate- $\epsilon$ -semialdehyde decarboxylase (ACMSD) plays a key role in tryptophan catabolism by enzymatically driving the production of PIC. Furthermore, NAD<sup>+</sup> is an important contributor to energy (ATP) production and plays a key role in the regulation of DNA repair, genomic stability, replication, and cell division. Most cancer cells have significantly higher energy consumption compared to non-transformed cells. The KP has been fully characterised in human neuroblastoma cells. However, no literature exists characterising the KP in the most aggressive form of brain cancer, astroglomas. Here we report the first characterisation of the KP in astrogloma cells using astrogloma cell lines (U87, U251 and SVG) and primary cultures of human foetal astrocytes (HFA) stimulated or not with IFN- $\gamma$  (100IU/ml). Reverse transcriptase (RT)-PCR revealed that astrogloma cell lines (n=6) expressed significantly lower ACMSD but higher IDO-1 compared to HFA. We also observed that astrogloma cell lines (n=6) produced significantly higher intracellular NAD<sup>+</sup> concentrations compared to HFA. These findings suggest that altered KP metabolism in astrogloma cell lines may promote tumour cell viability and ultimately contribute to tumour cell persistence. This study provides the foundation for the identification of novel therapeutic strategies that exploit the differences in KP metabolism we observed.

## POS-TUE-040

**PROPERTIES OF ISOLATED SKINNED FAST-TWITCH FIBRES FROM  $\alpha$ -ACTININ-3 KNOCKOUT MICE**

**Chan S.<sup>1</sup>**, Seto J.T.<sup>2,3</sup>, Houweling P.J.<sup>2</sup>, Yang N.<sup>2,3</sup>, North K.N.<sup>2,3</sup> and Head S.I.<sup>1</sup>

<sup>1</sup>School of Medical Sciences, University of New South Wales, Sydney, 2052 NSW, Australia. <sup>2</sup>Institute for Neuroscience and Muscle Research, The Children's Hospital at Westmead, Sydney, 2145 NSW, Australia. <sup>3</sup>Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, 2006 NSW, Australia.

$\alpha$ -Actinin-3 is found in the Z-disks of fast glycolytic skeletal muscle fibres, where it cross-links the actin filaments of the contractile apparatus. About 1 billion people worldwide are completely deficient in this protein. In this study we used individual skinned fibres from the EDL muscles of wild-type and *Actn3* knockout mice to examine possible mechanisms for the slowing of relaxation observed in  $\alpha$ -actinin-3-deficient whole muscle. Animals aged 9 to 10 months were sacrificed with an overdose of halothane (ethics approval UNSW). Mechanically skinned fibres were first placed in K<sup>+</sup>-HDTA solution containing low Mg<sup>2+</sup> (0.25 mM) and 30 mM caffeine, to deplete the SR of endogenous Ca<sup>2+</sup>, and 0.25 mM EGTA to chelate all released Ca<sup>2+</sup> and prevent SR Ca<sup>2+</sup> reaccumulation. The fibre was then reloaded with Ca<sup>2+</sup> for predetermined periods of time by exposure to a highly buffered Ca<sup>2+</sup> solution (pCa 6.57). Loading was rapidly terminated at the end of each loading period by a brief exposure to a relaxing solution, after which the fibre was washed in a K<sup>+</sup>-HDTA solution to remove excess EGTA. The fibre was then reexposed to the caffeine solution and the force response was recorded. The area under the force response curve was used as a measure of the amount of Ca<sup>2+</sup> released, and hence of the amount of Ca<sup>2+</sup> loaded. For all loading periods, the amount of Ca<sup>2+</sup> loaded by the SR, expressed as a percentage of the maximum amount it could load in our solution, was lower in knockout fibres than in wild-type fibres. This suggests that in knockout fibres the SR resequesters Ca<sup>2+</sup> at a slower rate than in wild-type fibres. This result provides one possible reason for the slowing of relaxation observed in whole *Actn3* knockout muscle. Following the SR loading experiments the fibre was chemically skinned and the properties of the contractile filaments were examined. Force-pCa and force-pSr curves were obtained by exposing the fibres to a series of increasing [Ca<sup>2+</sup>] and [Sr<sup>2+</sup>]. No differences were found between wild-type and knockout fibres in their pSr<sub>50</sub>-pCa<sub>50</sub>, indicating that the slowing of relaxation was not due to any shift in myosin heavy chain isoforms from fast types to slow-type. However, the knockout fibres had significantly steeper force-pCa curves than wild-type fibres (Hill coefficient 3.31  $\pm$  0.17 n=18 KO vs 2.68  $\pm$  0.07 n=17 WT, p=0.002). The impact of this on whole muscle relaxation times is unclear, but it does indicate that loss of  $\alpha$ -actinin-3 leads to subtle changes in the sensitivity of the contractile proteins to Ca<sup>2+</sup>.

## POS-TUE-041

# EXPLORING THE SPREAD OF EXCITATION THROUGHOUT THE TUBULAR NETWORK IN MAMMALIAN SKELETAL MUSCLE USING SUPERFAST CONFOCAL MICROSCOPY

Edwards J.N.<sup>1</sup>, Cully T.R.<sup>1</sup>, Thorn P.<sup>1</sup>, Gilbert D.G.<sup>2</sup> and Launikonis B.S.<sup>1</sup>

<sup>1</sup>School of Biomedical Sciences, The University of Queensland.

<sup>2</sup>Queensland Brain Institute, The University of Queensland.

In skeletal muscle, the rapid spread of excitation across the sarcolemma and throughout the tubular (t-) system is essential for uniform Ca<sup>2+</sup> release and subsequent force production. The longitudinal spread of excitation within the t-system network has been reported in spontaneously active mechanically skinned fibres. In such a large cell, every transverse tubule may not be excited following depolarization at the cell surface. Any longitudinal spread of excitation between sarcomeres where transverse tubules fail to depolarize cannot be easily measured with conventional imaging techniques. By imaging Ca<sup>2+</sup> transients with Oregon Green Bapta 5N at 15.5  $\mu\text{s line}^{-1}$  on a Zeiss 5 LIVE confocal system, we a) tracked the longitudinal spread of excitation along the t-system from the subsequently released Ca<sup>2+</sup> and b) also resolved the Ca<sup>2+</sup> release waveform with the highest temporal resolution to date. Following field stimulation of skinned fibres, we observed that in areas where transverse tubules failed to be excited by the initial stimulus, Ca<sup>2+</sup> release propagated in from the adjacent regions at a rate of  $\sim 16 \mu\text{m ms}^{-1}$  (n=6). The rise time of the Ca<sup>2+</sup> transient showed two phases. It initially rose rapidly for 1ms and then continued at a slowing rate for a further 0.5ms until the peak of the transient. Nav1.4 immunostaining identified a complex subsarcolemmal t-system network which may help ensure the synchronous spread of excitation throughout the fibre from the surface membrane. However, uniform calcium release in skeletal muscle also requires longitudinal tubules deep within the t-system network to pass action potentials between excited and 'failing' transverse tubules.

## POS-TUE-042

# OVEREXPRESSION OF HSP72 ATTENUATES SKELETAL MUSCLE PATHOPHYSIOLOGY IN MDX DYSTROPHIC MICE

Gehrig S.M.<sup>1</sup>, Van Der Poel C.<sup>1</sup>, Henstridge D.C.<sup>2</sup>, Schertzer J.D.<sup>1</sup>, Koopman R.<sup>1</sup>, Naim T.<sup>1</sup>, Febbraio M.A.<sup>2</sup> and Lynch G.S.<sup>1</sup>

<sup>1</sup>Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, Victoria, 3010, Australia. <sup>2</sup>Cellular and Molecular Metabolism Laboratory, Baker IDI, PO Box 6492, St Kilda Road Central, VIC, 8008, Australia.

An absence of dystrophin in muscle fibres results in fragility, membrane tears, Ca<sup>2+</sup> influx and an elevated cytoplasmic [Ca<sup>2+</sup>], resulting in the activation of degenerative pathways. Chronic degeneration and ineffective regeneration results in fibrotic infiltration leading to functional impairments in DMD patients and in muscles from dystrophin-deficient *mdx* mice. Heat-shock protein 72 (HSP72) has potential to protect contractile function and improve Ca<sup>2+</sup> handling in cardiac muscle. We tested the hypothesis that HSP72 overexpression would ameliorate the pathophysiology of skeletal muscles of *mdx* dystrophic mice. Contractile properties of isolated diaphragm muscle preparations from *mdx* mice overexpressing HSP72 (*mdx*<sup>HSP72</sup>) and *mdx* littermate control mice (n≥5) were determined according to methods we have described previously. HSP72 overexpression improved normalised force of isolated diaphragm muscle strips (P < 0.05), reduced collagen infiltration (P < 0.05), and improved the minimal Ferets variance coefficient, indicative of a reduced dystrophic muscle fibre pathology (P < 0.05). Serum creatine kinase levels were significantly lower in *mdx*<sup>HSP72</sup> mice compared with *mdx* littermate controls (P < 0.05), indicating a general reduction in muscle degeneration. The findings reveal that overexpression of HSP72 protein improved the dystrophic muscle pathology.

## POS-TUE-043

# ANTI-MUSK AUTOANTIBODIES CAUSE MUSK ACTIVATION AND INTERNALIZATION LEADING TO DISASSEMBLY OF ACETYLCHOLINE RECEPTOR CLUSTERS

Ghazanfari N.<sup>1</sup>, Gervasio O.L.<sup>1</sup>, Cole R.N.<sup>1</sup>, Ngo S.T.<sup>2</sup>, Reddel S.R.<sup>3</sup> and Phillips W.D.<sup>1</sup>

<sup>1</sup>Physiology & Bosch Institute, University of Sydney. <sup>2</sup>School of Biomedical Sciences, University of Queensland. <sup>3</sup>Dept of Molecular Medicine, Concord Hospital, Concord, NSW.

**Purpose:** Muscle Specific Kinase (MuSK) is a key component of a postsynaptic signaling complex required for induction of postsynaptic differentiation by agrin. MuSK has been recently identified as the auto-antigen in a subset of myasthenia gravis patients. Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular synapse that causes impaired neuromuscular transmission and muscle weakness. Recently, we have shown that injection of anti-MuSK-positive patient IgG into mice caused myasthenia gravis. Here we aimed to define the mechanisms by which anti-MuSK antibodies interfere with neuromuscular transmission. **Methods:** Immunoprecipitation was used to assess the effect of anti-MuSK antibodies on tyrosine phosphorylation of MuSK and AChR  $\beta$ -subunit. To investigate the effect of anti-MuSK antibodies on pre-existing AChR clusters, cultured mouse C2 myotubes were pre-treated with agrin (1nM, 4h) to form AChR clusters and then exposed to control human IgG or IgG from an anti-MuSK-positive MG patient. Confocal optical sections of control and experimental myotubes were used to compare the number and size of AChR clusters. To assess the effect of anti-MuSK antibodies on the subcellular distribution of MuSK, C2 myoblasts transfected with MuSK-GFP were imaged live on a confocal microscope. **Results:** Anti-MuSK antibodies caused tyrosine phosphorylation of MuSK and the AChR  $\beta$ -subunit, and internalization of MuSK (n=3). When added to cultured cells, anti-MuSK antibodies caused a significant reduction in number and size of AChR clusters compared with cells treated with control human IgG (n=3). **Conclusion:** anti-MuSK IgG may interfere with neuromuscular transmission by depleting MuSK from the postsynaptic membrane scaffold, leading to disassembly of AChR clusters.

## POS-TUE-044

# ORIGIN OF THE LOW-LEVEL EMG DURING THE CORTICAL SILENT PERIOD

Taylor J.L.<sup>1</sup>, Gandevia S.C.<sup>1</sup>, Herbert R.D.<sup>2</sup>, Petersen N.T.<sup>3</sup> and Butler J.E.<sup>1</sup>

<sup>1</sup>Prince of Wales Medical Research Institute and the University of New South Wales, Sydney. <sup>2</sup>University of Sydney, Sydney. <sup>3</sup>University of Copenhagen, Denmark.

The cortical silent period refers to a period of up to 300 ms of near silence in the electromyogram (EMG) after transcranial magnetic stimulation (TMS) over the motor cortex during voluntary contraction. Despite the name, there are often small amounts of EMG present. The origin of this activity is not known. We hypothesized that it arises through spinal reflex mechanisms, in which lengthening of the muscle during relaxation caused by the cortical silent period could cause muscle spindle firing and facilitate motoneurons. **Purpose:** The current study tested whether low-level EMG in the silent period depended on muscle lengthening. **Methods:** Subjects (n=8) performed maximal isometric, shortening and lengthening contractions of the elbow flexors during which TMS (90-100% stimulator output) was delivered over the motor cortex. The rate of elbow flexion during the shortening contraction (225 degrees/s) was chosen to offset the estimated muscle lengthening caused by muscle relaxation. Surface EMG activity was recorded from biceps brachii and brachioradialis muscles, and the low-level EMG during cortical silent periods produced by TMS was measured. **Results:** The low-level EMG activity in the cortical silent period was reduced by up to 60-65% in the shortening contraction compared to all other contraction conditions (p<0.05). There was no difference between the contraction conditions for the pre-stimulus EMG and the duration of the cortical silent period in both muscles was similar across the contraction conditions. **Conclusion:** Muscle lengthening contributes to the low-level EMG activity in the cortical silent period, probably through spinal reflex facilitation by muscle spindle afferents.

## POS-TUE-045

**REGENERATION EFFICIENCY IN AGED/SENESCENT SKELETAL MUSCLE DEPENDS ON THE TYPE OF INJURY**

Gunning P.W.<sup>1</sup>, Lee A.S.J.<sup>2</sup>, Joya J.E.<sup>2</sup>, Head S.I.<sup>3</sup>, Pather N.<sup>2</sup>, Anderson J.E.<sup>4</sup> and Hardeman E.C.<sup>2</sup>

<sup>1</sup>School of Medical Sciences, Pharmacology, University of New South Wales, Sydney, NSW. <sup>2</sup>School of Medical Sciences, Anatomy, University of New South Wales, Sydney, NSW. <sup>3</sup>School of Medical Sciences, Physiology, University of New South Wales, Sydney, NSW. <sup>4</sup>Faculty of Science, University of Manitoba, Winnipeg MB, Canada.

Studies have shown that regeneration is impaired in muscles of aged mice. Regeneration improves in aged muscle exposed to a young circulatory system indicating that muscle progenitor or satellite cells are intact (Conboy et al. 2005). We hypothesised that the efficiency of repair/regeneration in aged muscles may be due to the injury method and in particular, the extent of nerve and vascular damage. We subjected the extensor digitorum longus (EDL) muscles of young (3 months; n=7), aged (22 months; n=8) and senescent (27 months; n=10) female mice to myotoxin (notexin) injury, which leaves the basal lamina, associated blood vessels and nerves intact. We subjected young (n=6) and aged (n=7) EDL muscles to Denervation-Devascularization (DD) injury, which detaches the associated nerves and blood vessels. Notexin injury resulted in full regeneration with hyperplasia in young and hypertrophy in old and senescent muscles. In vitro contractility measurements showed comparable absolute and specific forces, and active stiffness between the untreated and regenerated young and senescent muscles. Both young and senescent muscles showed a significant increase in passive stiffness ( $p < 0.01$  One-Way ANOVA) and shift towards slow-twitch characteristics ( $p < 0.05$  student's t-test). With DD injury, there was a severe hypotrophy of myofibres and decrease in muscle size/weight in both young and aged regenerated muscles. The results clearly demonstrate that the nature of muscle injury determines the efficiency of muscle regeneration in both young and aged muscles.

## POS-TUE-047

**ROBUST STORE-OPERATED CALCIUM ENTRY IN AGED MAMMALIAN SKELETAL MUSCLE**

Edwards J.N.<sup>1</sup>, Gilbert D.F.<sup>2</sup>, Blackmore D.G.<sup>2</sup>, Murphy R.M.<sup>3</sup> and Launikonis B.S.<sup>1</sup>

<sup>1</sup>School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, 4072. <sup>2</sup>Queensland Brain Institute, The University of Queensland, Brisbane, QLD, 4072. <sup>3</sup>Dept of Zoology, La Trobe University, Melbourne, Vic, 3086.

Store-operated  $\text{Ca}^{2+}$  entry (SOCE) is a specialized mechanism in muscle, involving extracellular  $\text{Ca}^{2+}$  entry in response to depleting intracellular  $\text{Ca}^{2+}$  stores during work. Although a report recently suggested SOCE is compromised in aged muscle (Zhao et al, 2008, *Aging Cell*), we reassessed this with our novel, sensitive techniques (Launikonis & Rios, 2007, *J. Physiol*). Young (8-20 weeks) and aged (25 months) C57BL/10 mice from the same colony were compared for SOCE functionality and relevant protein abundances. Fluo-5N trapped in the tubular system of skinned fibres was imaged with confocal microscopy. Substitution of the standard intracellular solution with low  $\text{Mg}^{2+}$  solution induced  $\text{Ca}^{2+}$  release. There was initial  $\text{Ca}^{2+}$  uptake in sealed t-tubules, followed by depletion due to SOCE. SOCE deactivation followed  $\text{Ca}^{2+}$  reuptake into sarcoplasmic reticulum (SR) and reduction in myoplasmic  $\text{Ca}^{2+}$ . Robust SOCE was observed in all fibres (n=8) from aged mice. In some fibres, subsequent  $\text{Ca}^{2+}$  waves were observed with defined onset of SOCE, allowing determination of SOCE activation kinetics. Whilst SOCE activation was delayed in aged ( $38 \pm 3.1$  ms, n=4) compared to young ( $27 \pm 3.6$  ms, n=6,  $p = 0.044$ ) muscle, SOCE deactivation was robust. Of note, rate of SR refilling compared to rate of SOCE deactivation varied between aged fibres (n=8). Furthermore, Orai1 and Stim1 protein levels were also varied suggesting the need for physiological and biochemical measurements on the same aged fibres. We conclude that SOCE continues to work in aged muscle but its deactivation and activation thresholds, as well as the integral SOCE protein levels may vary.

## POS-TUE-046

**ISOTONIC FORCE DEPENDENT MUSCLE ACTIVITY IN THE MULTI-JOINT MOVEMENTS**

Yamauchi J.<sup>1</sup> and Ishii N.<sup>2</sup>

<sup>1</sup>Graduate School of Human Health Sciences, Tokyo Metropolitan University. <sup>2</sup>Graduate School of Arts and Sciences, The University of Tokyo.

To investigate how the muscular activity affects the dynamic properties during knee-hip extension movements, electromyography (EMG) activity was obtained from major working muscles in isometric and isotonic (force clamp) conditions. Nine healthy subjects performed various loads of concentric and isometric knee-hip extensions at their maximal effort on a servo-controlled dynamometer. EMGs were simultaneously recorded by using surface electrodes from seven muscles: the gastrocnemius (Gas), vastus medialis (VM), rectus femoris (RF), vastus lateralis (VL), biceps femoris (BF), semitendinosus (ST), and gluteus maximum (Glut). Also, EMGs of maximal voluntary isometric contractions for each muscle were measured and used for the normalization of the muscle activity during knee-hip extensions. The EMG was full-wave rectified, integrated and averaged with respect to time (mEMG). The force-velocity relation of the knee-hip extension movement was well described with a linear function ( $r = -0.996$ ). The EMG activities of VM and VL during maximum isometric knee-hip extension movement as compared with isolated muscle contractions were larger while RF was same; on the other hand, GA, BF, ST and GM were lower. In all muscles studies, mEMG did not change significantly with force in the isotonic condition. In VL, however, it was significantly smaller in isometric condition than in the lower forces of isotonic condition ( $P < 0.05$ ). In conclusion, the coordination between knee extensor and hip extensor muscles changes with force in the knee-hip extension movement. In particular, decreased activation of knee extensor relative to hip extensor under the large force may strongly affect the nature of force-velocity relation.

## POS-TUE-048

**MODULATION OF HUMAN CORTICOMOTOR EXCITABILITY BY SENSORY, MOTOR AND NOXIOUS STIMULI**

Chipchase L.S.<sup>1</sup>, Schabrun S.M.<sup>2</sup> and Hodges P.W.<sup>1,2</sup>

<sup>1</sup>School of Health and Rehabilitation Sciences, The University of Queensland, Brisbane, Queensland, Australia. <sup>2</sup>NHMRC Centre of Clinical Research Excellence in Spinal Pain, Injury and Health, The University of Queensland, Brisbane, Queensland, Australia.

**Background:** Electrical stimulation has been used as a means of inducing cortical plasticity in both healthy and pathological populations. However, the parameters used vary considerably and are often not consistent with those used in the clinic. In addition, it is unclear which parameters are the most beneficial for inducing directionally specific plastic change. Here, we aim to examine the effect of five clinically relevant electrical stimulation paradigms on corticomotor excitability. **Methods:** Transcranial magnetic stimulation was used to measure corticomotor excitability of the biceps brachii and triceps brachii muscles before and after 30 minutes of electrical stimulation. Ten healthy individuals received five different electrical stimulation paradigms, applied in a random order, to the right biceps brachii. Each electrical stimulation paradigm was delivered at least 3 days apart. Responses evoked by TMS were normalised to M-wave amplitudes. **Results:** Electrical stimulation (10Hz) delivered at sensory or noxious intensity produced a decrease in corticomotor excitability ( $p < 0.03$ ). In contrast, motor stimulation (30Hz) to create a functional muscle contraction resulted in a significant increase in corticomotor excitability ( $p = 0.04$ ) while stimulation at 10Hz sufficient to produce a motor twitch resulted in no significant change ( $p = 0.37$ ). Changes in cortical excitability were consistent across the agonist and antagonist muscles in all conditions ( $p > 0.19$ ). **Conclusion:** These results suggest that clinically relevant electrical stimulation paradigms can induce transient inhibitory and facilitatory changes in corticomotor excitability. The direction of excitability change may be dependent on the balance between positive and negative drives.



## POS-TUE-049

**A NOVEL REHABILITATION TOOL TO PROMOTE FUNCTIONAL RECOVERY AFTER STROKE**

**Mouawad M.R.**, Doust C.G., Max M.D. and McNulty P.A.  
Prince of Wales Medical Research Institute, Sydney 2031, Australia.

More than half of those who survive a stroke are profoundly disabled in activities of daily life. Because there is no cure for stroke, rehabilitation remains the only option to recover functional movement. In Australia there is no standardised approach to rehabilitation and no single proven therapy. We used the Nintendo Wii game system as an intensive therapy protocol. Seven stroke patients (mean age 65.3 years, 1-38 months post-stroke) and five healthy controls (mean age 59.0 years) undertook one hour of formal therapy on 10 consecutive weekdays. Additionally, the stroke patients gradually increased additional home practice to three hours per day. Functional testing was performed immediately pre- and post-therapy. Functional movement ability significantly improved for all seven patients. A significant decrease in the performance time for the Wolf Motor Function Test ( $p=0.006$ ) was matched by a significant improvement in Fugl-Meyer Assessment scores ( $p=0.013$ ). The improved functional test scores were reflected in activities of daily living, assessed using the Motor Activity Log ( $p=0.008$ ) and represents a direct transfer of the gains achieved in therapy to real-world tasks. The range of motion for upper limb joints increased on average by  $12.7^\circ$  and  $7.2^\circ$ , for passive and active movements respectively. No significant change was seen in any measure for the control subjects, despite an improvement in their skill level for the Wii therapy games. These results suggest that an intensive 2 week protocol using the Nintendo Wii is sufficient to induce significant and clinically relevant improvements in functional motor ability after stroke. Moreover the gains achieved in therapy translated to improved functional movement in activities of daily living.

## POS-TUE-050

**FEASIBILITY OF MEASURING VOLUNTARY ACTIVATION AFTER STROKE USING CORTICAL STIMULATION**

**Bowden J.L.** and McNulty P.A.  
Prince of Wales Medical Research Institute, Sydney 2031, Australia.

Maximal voluntary activation is a measure of the neural drive to produce maximal force. Impaired motoneurone drive is revealed by peripheral stimulation while cortical stimulation reveals impaired cortical output. Activation scores are calculated using the resting twitch which must be estimated for cortical stimulation. This study was designed to determine the feasibility of using cortical stimulation to study voluntary activation in stroke patients. Voluntary activation was studied in the elbow flexor muscles in 5 stroke patients (mean age 67 years; 1-16 years post-stroke) using both peripheral and cortical stimulation. Elbow flexion force was measured with an isometric myograph while electromyographic activity was recorded from biceps and triceps brachii. Stimuli were delivered to the peripheral nerve (0.1 ms pulse width) or to the motor cortex using transcranial magnetic stimulation (1 ms pulse) while subjects performed 5 sets of brief maximal and submaximal voluntary contractions (MVC). Peripheral voluntary activation scores for stroke patients in this study (median 77%) were lower than those reported in the literature for young, healthy subjects (97% Todd et al 2003). Similarly, the pattern of cortical activation in stroke patients was different and more inconsistent to that of healthy controls. Motor evoked potentials (MEPs) could not be evoked at rest in any stroke patient. In healthy controls the biceps normalised MEP was largest in contractions at 50% MVC, while in stroke patients this occurred most frequently at 75% MVC with a maximum occurring at 50% MVC in only 2 subjects. These results suggest that the methods used to measure voluntary activation using cortical stimulation in healthy subjects cannot be used with stroke patients.

## POS-TUE-051

**EXCITABILITY OF MOTOR CORTICAL OUTPUT TO HUMAN SCALENES MUSCLES IS ALTERED BY LUNG VOLUME**

**Butler J.E.**<sup>1</sup>, Hudson A.L.<sup>1</sup>, Taylor J.L.<sup>1</sup>, Anand A.<sup>2</sup> and Gandevia S.C.<sup>1</sup>  
<sup>1</sup>Prince of Wales Medical Research Institute and University of New South Wales, Sydney, Australia. <sup>2</sup>Vallabhbhai Patel Chest Institute, Delhi University, Delhi, India.

Pulmonary afferents are known to inhibit inspiratory output from the medulla with increasing lung volume. **Purpose:** The aim of this study was to assess the effect of pulmonary afferent feedback on the excitability of motor cortical output to the respiratory muscles. **Methods:** In 8 subjects lying supine, motor evoked potentials (MEPs) were recorded from the right scalenes muscles in the neck (obligatory inspiratory muscle) and from biceps (non-respiratory muscle). Pulmonary afferent feedback was altered by changing lung volume. Subjects performed two manoeuvres (10 trials each): (1) incremental inspiration from functional residual capacity (FRC) to total lung capacity (TLC) and (2) incremental exhalation from TLC to FRC. High-intensity transcranial magnetic stimulation (75-95% stimulator output) was delivered over the motor cortex during relaxation at three lung volumes; FRC, FRC + 40% inspiratory capacity, and FRC + 90% inspiratory capacity. Prior to stimulation, the breathing apparatus was closed so that subjects could relax at each volume. **Results:** The amplitude and area of the MEPs recorded from the scalenes muscles were ~ 50% greater at a high lung volume compared to lower lung volumes ( $p < 0.001$ ). However, there was no difference in MEP size for the same lung volume in inspiratory and expiratory manoeuvres. In the control muscle biceps, the size of MEPs was similar at all lung volumes and in the two manoeuvres ( $p \geq 0.2$ ). **Conclusion:** The results suggest that unlike their effect at the medulla, pulmonary afferents activated at high lung volume increase the excitability of the motor cortical output to inspiratory muscles.

## POS-TUE-052

**EFFECTS OF HIGH FREQUENCY AND THETA BURST TRANSCRANIAL MAGNETIC STIMULATION ON GRIP STRENGTH**

**Folmli B.** and Turman B.  
Faculty of Health Sciences & Medicine, Bond University.

Transcranial magnetic stimulation (TMS) involves a brief but strong magnetic field capable of activating cortical elements. With the introduction of repetitive TMS (rTMS) it has been possible to study the modulatory effects of various stimulation paradigms on the excitability of motor systems. The outcomes are representative of the diverse effects of rTMS dependant on both the intensity and frequency of stimulation (Houdayer et al. 2008). Most studies investigate these effects on the motor evoked potentials of resting or slightly active muscles. Although the influence of magnetic stimulation on maximum voluntary contraction force of quadriceps femoris muscle in healthy individuals have been reported (Urbach & Awiszus 2001), the upper extremity muscles have not been studied with different stimulation paradigms. In this study we examined the effects of 'simple' rTMS at 5Hz and theta burst stimulation paradigm (30Hz 3 pulse burst at 5Hz) on grip strength of healthy 18-35 yo individuals. An adjustable dynamometer (Jamar) was used to measure the grip strengths on Position-2 and Position-3 separations. Measurements were carried out before and after rTMS intervention with 300 pulses delivered to the forearm representation of motor cortex at 80% active motor threshold. Each participant was also subjected to sham stimulation in their first session. The preliminary results suggest that there is no consistent change in the grip strength with either stimulation paradigm. Houdayer, E., Degardin, A, Cassim, F. et al. Exp Brain Res (2008), 187: 207-217. Urbach, D. & Awiszus, F. Exp Brain Res (2001), 142: 25-31.

## POS-TUE-053

**ARE RAT MODELS OF RUBROSPINAL TRACT INJURY ANY GOOD TO ASSESS RECOVERY OF HAND FUNCTION?**

**Morris R.**, Tosolini A.P., Batten N.D. and Goldstein J.D.  
School of Medical Sciences, UNSW.

The rubrospinal tract (RST) has recently been the focus of considerable attention in the field of spinal cord injury and repair. Lesions damaging variable extents of the LF but that consistently disrupt the RST support the view that the RST is involved in the control of the paw/digit movements while reaching. Such lesions, however, also damage other pathways within the LF that possibly contribute to reaching. The present study was designed to isolate more precisely the contribution of the RST to skilled movements of the forelimb. Rats (N=21) were trained on the skilled reaching task and were subsequently subjected to either 1) large lesions of the LF that extend below the central canal (CC), 2) medium lesions of the LF, restricted to its dorsal part and that stop at the level of the CC or 3) small lesions of the LF, restricted to its dorsal part with considerable sparing above the CC. All lesions included the full extent of the RST and care was taken in all instances to leave the dorsal roots intact (Wu et al., 2009). Detailed movement analysis revealed that, although all operated animals were still able to reach, the three types of lesions differently affected some components of the reach. Most interestingly, however, the pronation and arpeggio movements were impaired or missing to the same extent in all groups of lesions, suggesting that these components of the reach are under the control of the RST. The results are discussed in terms of translation of animal models of cervical spinal cord injury to clinically relevant therapeutic scenarios to improve the quality of life of people living with quadriplegia.

## POS-TUE-055

**BEHAVIOUR OF HUMAN GENIOGLOSSUS SINGLE MOTOR UNITS (SMU) DISCHARGE PROPERTIES IN QUIET BREATHING, CO<sub>2</sub> AND CPAP**

**Saboisky J.P.**<sup>1</sup>, Eckert D.J.<sup>1</sup>, Jordan A.S.<sup>2</sup>, Trinder J.A.<sup>2</sup>, White D.P.<sup>1</sup> and Malhotra A.<sup>1</sup>  
<sup>1</sup>Harvard University, Brigham and Women's Hospital, Boston, Massachusetts, USA. <sup>2</sup>University of Melbourne, Victoria, Australia.

The genioglossus is a primary muscle involved in dilating the upper airway. Two primary stimuli that contribute to genioglossal control are CO<sub>2</sub> and negative pressure which can modify the behavior of respiratory drive through chemoresponsiveness and mechanoreceptor activation respectively. We examined genioglossus SMU discharge properties to quantify neural drive during periods of quiet breathing, elevated ET-CO<sub>2</sub> and CPAP (2cmH<sub>2</sub>O increments until 10cmH<sub>2</sub>O) to reduce negative pressure influences on muscle activity. 15 subjects studied awake lying supine, breathing through a nasal mask. 3 fine-wire electrodes were inserted after ultrasound. We measured onset time, onset and peak firing frequency relative to respiration for 96 SMUs, tracked throughout 8 conditions. Genioglossus SMU activity increased with CO<sub>2</sub>, there was an increased discharge rate of inspiratory units (19Hz to 21Hz, p<0.05) with activation earlier in the inspiratory cycle (7.5%TI to -9.1%TI, advancement p<0.05), and firing for a longer period of the respiratory cycle (80.5%TI to 105.7%TI, p<0.05). An additional 33.3% of distinguishable SMUs within the selective electrode recording area were recruited. CPAP led to a progressive inhibitory response on the number of motor units active. At ~6cmH<sub>2</sub>O a similar number of motor units were active as in baseline conditions, with peak frequencies of the inspiratory units returned to baseline 19.3Hz. At 10cmH<sub>2</sub>O the number of active units was 36.1% less than baseline conditions. Genioglossus SMU activity is altered in response to chemical and mechanical stimuli. Inspiratory Phasic and Inspiratory Tonic SMUs have earlier pre-activation and increased peak firing frequencies during inspiration in response to CO<sub>2</sub>, and this increase in activity is terminated by CPAP.

## POS-TUE-054

**NEURONS IN THE LOCUS COERULEUS AND SUBCOERULEUS NUCLEUS THAT PROJECT TO SPINAL CORD ARE NOT NORADRENERGIC**

**Liang H.**<sup>1</sup>, Watson C.<sup>2</sup> and Paxinos G.<sup>1</sup>

<sup>1</sup>Prince Of Wales Medical Research Institute, PO Box 82, St Pauls, NSW 2031. <sup>2</sup>Curtin University of Technology, Perth, Australia.

The locus coeruleus and subcoeruleus nucleus play an essential role in arousal. About 95% of the noradrenaline in the brain is in the neurons of the locus coeruleus. Descending projections from the nucleus locus coeruleus and subcoeruleus to the spinal cord have been demonstrated in a number of mammals, and it has been assumed that the spinal projecting neurons are noradrenergic. We studied this projection in the C57BJ6 mouse by injecting the retrograde tracer Fluoro-gold into the cervical spinal cord. We found that spinal projecting neurons in the nucleus locus coeruleus were confined to the ipsilateral side of the injection, but the subcoeruleus nucleus had bilateral labelling with an ipsilateral predominance. Double labelling with anti-tyrosine hydroxylase (TH) revealed that not all of the spinal projecting neurons were TH positive. We have concluded that the TH-negative projecting neurons in the locus coeruleus and subcoeruleus nucleus are not noradrenergic. This suggests that there are at least two distinct cell populations in the locus coeruleus - a finding which is consistent with the involvement of this nucleus physiological functions other than arousal and sleep modulation.

## POS-TUE-056

**THE BOUNDARIES AND CONTENTS OF THE MAMMALIAN ISTHMUS REVEALED BY FGF8 GENE EXPRESSION**

**Watson C.R.**<sup>1,2</sup>

<sup>1</sup>Prince of Wales Medical Research Institute, Sydney NSW. <sup>2</sup>Curtin University, Perth WA.

The isthmus is a distinct part of the vertebrate brain, situated between the midbrain and the first rhombomere. The signature component of the vertebrate isthmus is the trochlear nucleus. Transient Fgf8 expression in the isthmus region plays a crucial role as a secondary organizing center in the embryonic brain. In avian and mammalian embryos, Fgf8 is expressed immediately caudal to the junction of Otx2 and Gbx2 expression. The isthmus is recognized as a substantial part of the hindbrain of adult birds and reptiles, but most studies on mammals ignore its existence entirely. **METHOD** This study has defined the contents and boundaries of the mammalian isthmus by examining the distribution of cells of the Fgf8 lineage in postnatal mice (n=4) possessing an Fgf8-cre-lacZ transgene (stained sections kindly provided by Dr Tomomi Shimogori of RIKEN). **RESULTS** The neuron groups expressing Fgf8 correspond well to the predictions based on avian studies; the trochlear, dorsal raphe, caudal linear, and parabigeminal nuclei are all intensely labelled with lacZ. A large part (perhaps 50%) of the substantia nigra (SN) appears to be derived from the isthmus. Only a small portion of SN can therefore be considered to be mesencephalic, since the rostral parts of SN belong to the diencephalon. The Fgf8-cre preparations also indicate that the whole cerebellar vermis is derived from the isthmus. **CONCLUSION** This study demonstrates the isthmus character of many structures at the midbrain-hindbrain junction, and point to the need for a re-evaluation of the regional anatomy of the substantia nigra. These results demand recognition of the existence of the isthmus as a major component of the mammalian hindbrain.

## POS-TUE-057

**AFFERENTS TO THE MESENCEPHALIC RETICULAR FORMATION**Qi Y.<sup>1</sup>, Paxinos G.<sup>1,2</sup> and Watson C.<sup>1,3</sup><sup>1</sup>Prince of Wales Medical Research Institute, The University of New South Wales, NSW 2031, Australia. <sup>2</sup>School of Medical Science, The University of New South Wales, NSW 2052, Australia. <sup>3</sup>Faculty of Health Science, Curtin University, Perth, WA 6845, Australia.

Studies on the mesencephalic reticular formation (mRt) in the 1970s and 1980s indicated that this structure was involved in many behaviors, including eye movement, eating, sleep-walking, urination, defecation and sexual activity. However, the anatomy of this region has not been well defined. We found a distinct group of calbindin positive neurons in the central mRt. In order to map input to these neurons, we injected the retrograde tracer (FluoroGold; 20 nanolitres per rat) into the mRt. The mRt receives afferents from diverse regions in the brain. The major sources of forebrain afferents are the internal pyramidal layer (V) of the motor cortex (the primary and secondary), the primary somatosensory cortex, the insular cortex, the amygdala, the medial preoptic nucleus, the zona incerta, the ventromedial hypothalamic nucleus, the paraventricular hypothalamic nucleus and the lateral hypothalamic area. The mRt also receives substantial input from the periaqueductal grey, the superior colliculus, the substantia nigra, the dorsal tegmental nucleus, the superior olivary complex, the spinal trigeminal nucleus, and the lateral paragigantocellular nucleus. The most of the structures that send input to the mRt are related to motor activity, which is consistent with the physiological literature on the role of the mRt in patterned motor activity.

## POS-TUE-058

**THE ARCUATE NUCLEUS OF THE HINDBRAIN IS A DISPLACED INFERIOR OLIVARY COMPONENT**Fu Y.H.<sup>1</sup>, Paxinos G.<sup>1,2</sup> and Watson C.<sup>1,3</sup><sup>1</sup>Prince of Wales Medical Research Institute, The University of New South Wales, NSW 2031, Australia. <sup>2</sup>School of Medical Science, The University of New South Wales, NSW 2052, Australia. <sup>3</sup>Faculty of Health Science, Curtin University, Perth, WA 6845, Australia.

The arcuate nucleus is a prominent cell group in the human hindbrain, and is considered to be a precerebellar nucleus. It is not commonly found in other mammals, and it has not been previously identified in the mouse. To further investigate whether the arcuate nucleus issues mossy fibers to the cerebellum like the lateral reticular nucleus, or whether it can be grouped developmentally and functionally with the cells of the inferior olive, the only source of climbing fibers in the cerebellar cortex, we examined the cytology, gene expression, immunohistochemistry, and cerebellar projections of the arcuate nucleus, the inferior olive, and the lateral reticular nucleus in the mouse (n=8). Gene expression was examined using the AGEA tool (<http://mouse.brain-map.org>). We found that the arcuate nucleus is a distinct but inconstant group of neurons, which lie either on the ventral surface of the pyramid or in a stream continuous with the ventrolateral part of rostral inferior olive, the principal nucleus. The neurons of arcuate nucleus and of inferior olive share three characteristics: they both stain strongly for calbindin; they both express a number of genes not seen in the lateral reticular nucleus, such as Htr5b, Tmem16B, and Stac; they both project to the contralateral cerebellum. We conclude that the arcuate nucleus is a subgroup of the inferior olive, issuing climbing fibers to cerebellum, but that during the caudal extension of the pyramidal tract, its cells are separated from the remaining inferior olive.

## POS-TUE-059

**BISTABILITY PERMITS RAPID TRANSITIONS BETWEEN SPATIOTEMPORAL WAVES AND SYNCHRONOUS SPIKING ACTIVITY IN A COMPUTATIONAL MODEL OF CORTEX**Heitmann S.<sup>1,2</sup>, Gong P.<sup>3,4</sup> and Breakspear M.<sup>1,2,5,6</sup><sup>1</sup>School of Psychiatry, The University of New South Wales, Australia. <sup>2</sup>Black Dog Institute, Sydney, NSW, Australia. <sup>3</sup>School of Physics, The University of Sydney, NSW, Australia. <sup>4</sup>Faculty of Medicine, The University of Sydney, NSW, Australia. <sup>5</sup>Queensland Institute of Medical Research, Brisbane, QLD, Australia. <sup>6</sup>Royal Brisbane and Women's Hospital, QLD, Australia.

Rapid changes in behaviour must be driven by rapid changes in dynamic brain states, yet the brain must also remain stable in the face of noise and uncertainty. The neural mechanism for satisfying these competing demands is unknown and presents a fundamental problem to computational neuroscience. We modelled a small region of motor cortex as an ensemble of phase-coupled neural oscillators arranged in a two-dimensional sheet. The strengths of the lateral connections between neurons (synaptic kernels) in this sheet varied periodically with distance. The model induced either phase-matched synchronous firing or spatiotemporal wave-like firing patterns across the entire neural ensemble. Increasing the baseline strength of neural connections favoured synchronous firing patterns whereas increasing the amplitude of the periodicity favoured wave patterns. Manipulating both parameters, we found that certain combinations of baseline strength and periodicity gave rise to bistability wherein the neural ensemble converged to either synchrony or waves depending on initial conditions. We argue that wave patterns and synchronous firing patterns represent distinct stable brain states and that switching between these brain states is consistent with switching between behavioural states. We suggest that very rapid transitions between otherwise stable brain states is most efficient within the region of bistability and that motor cortex may exploit this property to achieve rapid transitions between motor behaviours.

## POS-TUE-060

**PATHOGENIC ROLE OF GENE MUTATIONS IN ALS**Warraich S.T.<sup>1,3</sup>, Durnall J.C.<sup>1</sup>, Williams K.L.<sup>1</sup>, Yang S.<sup>1</sup>, Thoeng A.D.<sup>1,2</sup>, Nicholson G.A.<sup>1,3,4</sup> and Blair I.P.<sup>1,3</sup><sup>1</sup>Northcott Neuroscience Lab, ANZAC Research Institute, Sydney, NSW, Australia. <sup>2</sup>Department of Physiology, University of Sydney, NSW, Australia. <sup>3</sup>Faculty of Medicine, University of Sydney, NSW, Australia. <sup>4</sup>Molecular Medicine Laboratory, Concord Hospital, Sydney, NSW, Australia.

**BACKGROUND:** ALS (amyotrophic lateral sclerosis) is an adult-onset neurodegenerative disorder that causes degeneration of both upper and lower motor neurons. The principal pathology of ALS is the presence of ubiquitin positive protein aggregates in the cell body of the motor neurons. TDP-43, principally a nuclear protein (encoded by TARDBP gene) is a major component of the Ubiquitinated inclusions in ALS. Several TARDBP and FUS mutations have recently been reported in ALS cases. Mutations in FUS are the second most common known gene abnormality in familial ALS after SOD1. **OBJECTIVES:** The purpose of this study is to screen for additional mutations in TARDBP and FUS genes among familial ALS cohort (n=124) and sporadic ALS (n = 247). Another aim is to establish neuronal cell models expressing mutantTDP-43 and FUS. The effects of TARDBP mutations are also being investigated in lymphoblasts. **RESULTS:** Three TARDBP gene mutation were found in the ALS cohort. One new FUS mutation R521H was found in the extended FALS cohort. Our preliminary immunohistochemistry and immunofluorescence results show that there is presence of aggregates and an abnormal redistribution of TDP-43 from nucleus to the cytoplasm in cells transfected with TARDBP mutations when different cellular stresses are induced. These phenotypic changes under different stresses implicate pathways and mechanisms through which TDP-43 plays a pathogenic role. Preliminary results of induced cellular stresses on patient lymphoblasts show an increase in proteolytic cleavage and also redistribution of TDP-43 from nucleus to the cytoplasm.



## POS-TUE-061

# **EXPRESSION OF VOLTAGE-GATED SODIUM CHANNEL $\beta$ -SUBUNITS IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

**Zhong W.**, Kerr M.L., Noakes P.G. and Bellingham M.C.  
School of Biomedical Sciences, University of Queensland, Brisbane, 4072 QLD, Australia.

**BACKGROUND:** Motoneuron hyper-excitability and increased persistent  $\text{Na}^+$  current ( $I_{\text{NaP}}$ ) is an early feature of amyotrophic lateral sclerosis (ALS) in both humans and transgenic mouse models of ALS (hSOD1<sup>G93A</sup>). We previously reported that expression of voltage-gated sodium channel ( $\text{Na}_v$ )  $\alpha$ -subunit genes were up-regulated in hSOD1<sup>G93A</sup> mouse motor cortex during the first postnatal week.  $\text{Na}_v$  channel activity can also be modulated by associated  $\beta$ -subunits. Here, we investigated levels of gene expression of  $\text{Na}_v$   $\beta$ -subunits in motor cortex, brainstem and lumbar spinal cord of wild type (WT) and hSOD1<sup>G93A</sup> mice aged P0, P7, P16, P28 and P71. **METHODS:** Samples were dissected from these three brain regions of mice anaesthetised with sodium pentobarbitone (100 mg/kg i.p.) (n=3-9 for each age and genotype), followed by total RNA extraction and reverse transcription. mRNA abundance was measured by real-time PCR using commercial TaqMan probes for mouse  $\text{Na}_v$   $\beta$ 1,  $\beta$ 2,  $\beta$ 3 and  $\beta$ 4. Data were normalized to the geometric mean of multiple reference genes (Ubc, Hprt1 and Sdha). **RESULTS:** In WT mice,  $\text{Na}_v$   $\beta$ 1,  $\beta$ 2 and  $\beta$ 4 expression levels were very low at P0 in cortex, brain stem and lumbar spinal cord.  $\beta$ 1 expression increased for three weeks after birth and then stabilized, while  $\beta$ 2 and  $\beta$ 4 expression remained at low levels throughout the postnatal period.  $\beta$ 3 expression was highest at birth in all areas, relative to expression of other  $\beta$ -subunits, then declined rapidly to stable moderate levels at P16. In hSOD1<sup>G93A</sup> mice, expression patterns of  $\beta$ -subunits in all regions were similar to WT littermates; only  $\beta$ 3 expression was significantly increased by 1.36 fold at P7 in hSOD1<sup>G93A</sup> cortex and by 1.75 fold at P21 in hSOD1<sup>G93A</sup> spinal cord, compared with WT controls. **CONCLUSION:**  $\beta$ 3 subunit gene expression levels were up-regulated postnatally in some hSOD1<sup>G93A</sup> mouse brain areas; this may contribute to neuronal hyper-excitability due to increased  $I_{\text{NaP}}$ .

## POS-TUE-063

# **NEURAL SST<sub>1</sub> AND SST<sub>2</sub> RECEPTORS DECREASE CHLORIDE SECRETION IN THE GUINEA-PIG SMALL INTESTINE**

**Foong J.P.P.<sup>1</sup>**, Parry L.J.<sup>2</sup> and Bornstein J.C.<sup>1</sup>

<sup>1</sup>University of Melbourne Department of Physiology Parkville Vic 3010.

<sup>2</sup>University of Melbourne Department of Zoology Parkville Vic 3010.

**Purpose:** Somatostatin (SOM) inhibits secretion. We have found that vasoactive intestinal peptide (VIP) secretomotor neurons display inhibitory postsynaptic potentials that are mediated by SOM acting on SST<sub>1</sub> and SST<sub>2</sub> receptors. This study investigated the role these SST receptors play in secretion in guinea-pig small intestine. **Methods:** Mucosa-submucosa preparations were dissected and mounted in Ussing chambers to measure  $\text{Cl}^-$  secretion across the mucosa. All drugs were added serosally. Veratridine (1  $\mu\text{M}$ ) was applied to stimulate neurons and provide a robust secretory response, reflected by an increase in short circuit current ( $\Delta I_{\text{sc}}$ ). Quantitative-PCR (qPCR) was performed on stripped guinea-pig submucous plexus and mucosa to provide a relative quantification of SST<sub>1</sub> and SST<sub>2</sub> receptor gene expression, using ribosomal 18S as the endogenous house-keeping gene. Results were considered significant if  $P < 0.05$ . **Results:** SOM (50nM) induced a tetrodotoxin (1  $\mu\text{M}$ )-sensitive (n=6), decrease in basal secretion (n=9). SOM also significantly reduced veratridine-induced  $\Delta I_{\text{sc}}$  (n=8). The effects of SOM were significantly reduced by blocking SST<sub>1</sub> (SRA 880 3  $\mu\text{M}$ ; basal secretion n=8, veratridine-induced  $\Delta I_{\text{sc}}$  n=9) and SST<sub>2</sub> receptors (CYN 154 806 100nM; basal secretion n=10, veratridine-induced  $\Delta I_{\text{sc}}$  n=10) individually, or in combination (basal secretion n=6, veratridine-induced  $\Delta I_{\text{sc}}$  n=7). Blocking SST<sub>1</sub> receptors were more effective than blocking SST<sub>2</sub> receptors. q-PCR demonstrated that SST<sub>1</sub> (n=6) and SST<sub>2</sub> (n=6) receptors were highly expressed in the submucous plexus compared to the mucosa, and SST<sub>1</sub> receptor expression was significantly higher than SST<sub>2</sub>. **Conclusions:** SOM exerts its antisecretory effects indirectly by suppressing the excitability of VIP secretomotor neurons via SST<sub>1</sub> and SST<sub>2</sub> receptors. SST<sub>1</sub> receptors appear to play a more prominent role in secretion.

## POS-TUE-062

# **CO-ORDINATING CONTRACTION IN THE PREGNANT UTERUS**

**Davies M.**, Tonta M.A., Li Q., Iqbal J., Sheehan P.M., Coleman H.A., Lang R.J. and Parkington H.C.  
Dept Physiology, Monash University, Melbourne, Vic 3800, Australia.

Uterine contractions require  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels, raising the critical question as to events mediating the depolarization necessary for opening these channels. In gut, Interstitial cells of Cajal (ICC) play a key role in pacemaking. We investigated the possibility that ICC-like cells underpin uterine contractions. ICC were stained with vimentin and smooth muscle (SM) cells localized with SM actin in uteri from 20 women undergoing caesarean delivery. Uteri from 23 pregnant mice were also studied. Contractions were recorded simultaneously with membrane potential, using intracellular microelectrodes. Ionic currents were recorded via conventional patch clamp, the characterized cells were then subjected to single cell PCR. In human tissue, ICC occupied  $1.3 \pm 0.3\%$  of SM bundles, but occupied  $2.6 \pm 0.4\%$  of the space between bundles ( $p = 0.01$ ).  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  channels were rare in ICC (maximum current  $58 \pm 10 \text{ pA}$ ) (facilitating excitability), but abundant in SM cells ( $904 \pm 163 \text{ pA}$ ) (facilitating quiescence). This was confirmed using single-cell PCR. In mouse uterus, ICC staining was absent from longitudinal (LSM) layer, was present in circular (CSM) layer and abundant between the two layers. Vimentin-staining co-localized with c-Kit staining. LSM cells had membrane potentials of  $-68 \pm 1 \text{ mV}$  and were quiescent, while CSM had membrane potentials of  $-55 \pm 1 \text{ mV}$  ( $p = 0.008$ ) and had spontaneous contractions. The c-Kit antagonist imatinib did not abolish spontaneous activity in CSM but it blocked the spread of activity, generated in CSM, to LSM. We conclude that ICC are not responsible for generating contractions in uterus but may be involved in propagation of activity. Human uterus consists of an intricate network of thin bundles of SMC. Efficient communication between bundles is critical for coordinated organ contraction. Targeting uterine ICC could have therapeutic possibilities.

## POS-TUE-064

# **INNERVATED MYOFIBROBLASTS IN THE URINARY BLADDER? FUNCTIONAL AND ULTRA-STRUCTURAL EVIDENCE**

Sandow S.L., Sadananda P. and Burcher E.

Dept of Pharmacology, School of Medical Science, University of New South Wales, Sydney, NSW 2052.

We have shown that neurokinin A (NKA) and other agents are able to contract isolated mucosal strips from porcine bladder (Sadananda et al., 2008). Dense smooth muscle actin staining on the discrete population of porcine suburothelial myofibroblasts suggested that these cells may be contractile, and mediate the contraction to NKA, hypothesised to be originate from suburothelial afferent nerves. The purpose of this study was to examine the ultrastructure of the porcine bladder mucosa and characterize the relationship of the suburothelial myofibroblasts with adjacent cells. Pig bladders (n=2) were obtained from an abattoir, fixed in glutaraldehyde and processed for electron microscopy. The suburothelial population of porcine bladder myofibroblasts were morphologically similar to the deep myofibroblasts of human detrusor, in that they have prominent rough endoplasmic reticulum and elongated processes. Extensive nerve bundles with varicosities were located below the urothelium and in close proximity to myofibroblasts. These nerve bundles contained individual varicosities with discrete regions (bare of Schwann cells) containing accumulations of synaptic vesicles, which faced the potential neuroeffector cell. This is in accordance with the definition of neuroeffector junctions, as neurotransmitter release sites. Further, the dense-cored synaptic vesicles in these nerves were 100-250 nm in diameter, consistent with a primarily peptidergic content. In low resolution studies (n=4), synaptophysin and tachykinin immunoreactivity indicated that nerve fibres containing tachykinin were located within the suburothelial nerve plexus and in association with myofibroblasts. The strategic location of suburothelial myofibroblasts between the urothelium and afferent nerve plexus suggests that they are involved in sensory processing and cross talk between the two cell layers. Data suggest that peptides such as NKA are released from these dense-cored synaptic vesicle-containing varicosities, to act directly on suburothelial myofibroblasts, resulting in contraction of the porcine bladder mucosa. Subsequent studies will further characterise this hypothesis. Sadananda P, Chess-Williams R & Burcher E. (2008). Br J Pharmacol 153, 1465-1473.

## POS-TUE-065

**EFFECTS OF A GHRELIN RECEPTOR AGONIST AT THE SPINAL CORD LEVEL ON URINARY BLADDER CONTRACTILE ACTIVITY**

**Ferens D.<sup>1</sup>**, Yin L.<sup>1</sup>, Ohashi-Doi K.<sup>1</sup>, Habgood M.<sup>2</sup>, Gale J.<sup>3</sup> and Furness J.B.<sup>1</sup>

<sup>1</sup>Department of Anatomy and Cell Biology, University of Melbourne, Australia. <sup>2</sup>Department of Pharmacology, University of Melbourne, Australia. <sup>3</sup>Clinical Research, Pfizer Global R & D, Sandwich Laboratories, Kent, United Kingdom.

We have previously shown that ghrelin receptor (GhrR) agonists can act on the spinal cord to selectively stimulate autonomic outputs to blood vessels and the colorectum, whilst not affecting small intestine or heart. We have now investigated whether spinal pathways controlling the urinary bladder could also be affected by GhrR agonists. **Method:** A new potent GhrR agonist, capromorelin, was injected either intravenously (10mg/kg) or intrathecally (250µg) into Sprague Dawley rats (n=10) undergoing continuous infusion cystometry under urethane anesthesia. Two rats were used to test the effect of capromorelin directly on detrusor muscle with 6 strips set up in organ baths. A series of rats (n=14) was perfused following stimulation of bladders for 30 minutes to induce c-Fos expression within the spinal cord that was later co-localized with GhrR gene expression. **Results:** Both i.v. and i.t. capromorelin caused increased, but disrupted, contractile activity, without improving efficiency of voiding. The effect was blocked by both hexamethonium and atropine. Direct application of capromorelin (up to 100µM) had no effect on bladder contractility. Immunohistochemistry revealed the early gene product c-Fos in neurons of the autonomic medio-lateral cell columns at L5 to S2, with 20% also expressing the GhrR gene transcript. **Conclusions:** We conclude that ghrelin receptors are expressed in lumbosacral autonomic preganglionic neurons that control excitatory pathways to the detrusor muscle.

## POS-TUE-066

**c-FOS IDENTIFICATION OF SPINAL NEURONS ACTIVATED BY THE URETHRO-GENITAL REFLEX IN FEMALE GUINEA-PIGS**

**Yuan S.Y.**, Vilimas P.I., Zagorodnyuk V.P. and Gibbins I.L.

Centre for Neuroscience, Flinders University, Adelaide, SA, Australia.

To examine spinal circuits underlying the urethro-genital reflex (UGR), we visualised neurons activated by the reflex with c-Fos immunoreactivity in the lumbar and sacral spinal cord of female guinea-pigs. In anaesthetised female guinea pigs (200-250g), a balloon was inserted into the urethra and inflated for 30s every 10 minutes for 90 minutes to activate UGR. A second balloon was inserted into the vagina and uterus to record reflex contractions. One control group had no balloon; a second control group had a vaginal recording balloon only. In some animals, the spinal cord was transected acutely at L4 or T12 segments. Animals were left for 90 minutes after eliciting the UGR and were then processed for c-Fos immunoreactivity in L3 to S2 segments of the spinal cord. Using multivariate analysis of variance, there was no significant difference in spinal c-Fos expression between the two control conditions (n=5 each). The UGR significantly increased the number of c-Fos-expressing neurons by 100-200% throughout dorsal and intermediate laminae at S2 but only by about 100% in superficial laminae at L3 (n=5). Transection at T12 had little consistent effect on c-Fos expression in response to UGR at either spinal level (n=5). However, transection at L4 significantly increased the number of c-Fos-expressing neurons in intermediate laminae by about 100% at S2 and by about 50% at L3 in response to UGR (n=5). These data suggest that the UGR apparently activates local spinal inhibitory circuits that suppress expression of c-Fos in neurons likely to include preganglionic outputs to the pelvic viscera.

## POS-TUE-067

**DISRUPTED GASTRIC SLOW WAVE ACTIVITY AND NEUROMUSCULAR TRANSMISSION IN A MURINE MODEL OF TYPE 2 DIABETES**

**McDonough K.J.** and **Beckett E.A.H.**

Discipline of Physiology, School of Medical Sciences, Faculty of Health Sciences, University of Adelaide, South Australia 5005.

Gastric motor function is frequently disordered in patients with type 2 diabetes mellitus (T2DM) and can be associated with symptoms such as nausea and bloating, impaired absorption of nutrients and medications, and disordered blood glucose control. Delayed gastric emptying has traditionally been attributed to autonomic neuropathy however a weak correlation between autonomic nerve damage and the extent of disordered motility suggests other mechanisms are affected. In addition, acute variations in blood glucose have major effects on gastric emptying but the mechanisms are poorly understood. In this study, slow wave activity and postjunctional neural responses were compared in non-diabetic and T2DM (db/db) gastric tissues in normoglycaemic and hyperglycaemic conditions. In normoglycaemic conditions db/db antral slow waves were of smaller amplitude ( $14.1 \pm 1.0$  mV) and higher frequency ( $10.5 \pm 0.6$  events.min<sup>-1</sup>) than in non-diabetics ( $22.9 \pm 2.2$  mV and  $5.1 \pm 1.0$  events.min<sup>-1</sup> respectively; n=7). In non-diabetic antrum acute elevation of glucose concentration to 33mmol produced a decrease in slow wave rate (to  $4.0 \pm 1.0$  events.min<sup>-1</sup>) and increased the variability of slow wave intervals. In contrast, the amplitude, frequency and variability of slow waves in db/db antrum were unchanged by acute hyperglycaemic conditions. Inhibitory junction potentials, evoked by multiple pulses of electrical field stimulation (0.5ms pulse duration, 5-20Hz) were significantly attenuated in db/db fundus. Kit-immunohistochemistry revealed reduced densities of both intramuscular ICC (ICC-IM) and NOS-positive inhibitory neurons in db/db gastric fundus compared to non-diabetic fundus. These pathophysiological changes to gastric slow wave rhythm and neuromuscular transmission likely contribute to the disrupted gastric motility patterns and impaired accommodation to stomach filling associated with T2DM.

## POS-TUE-068

**SLOWLY PROPAGATING MOTOR ACTIVITY IN THE ISOLATED RABBIT SMALL INTESTINE**

**Costa M.<sup>1</sup>**, Spencer N.<sup>1</sup>, Hennig G.<sup>2</sup> and Brookes S.J.H.<sup>1</sup>

<sup>1</sup>Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, South Australia. <sup>2</sup>Department of Physiology & Cell Biology, University of Nevada, USA.

In addition to spontaneous myogenic activity, generated by the pacemaker net of interstitial cells of Cajal, and neurally mediated content dependent movements, in conscious animals spontaneous neural activity generates slowly migrating motor complexes. It has not been possible to establish how these three separate mechanisms of motor activity interact to produce the complex adaptive intestinal movements, because they do not occur together in isolated preparations. Purpose: To investigate if migrating motor complexes can be generated in isolated segments of intestine. Methods: We investigated the spatio-temporal features of motor activity in isolated segments of intestine taken from 8 albino rabbits (killed by iv lethobarbital), cannulated and placed in a bath of oxygenated Krebs solution at 37 °C. Spatio-temporal maps of changes in diameter were constructed from video recordings (1). Results: Spontaneous pendulum activity of the longitudinal muscle generated aborally propagating contractions readily visible in the spatio-temporal maps (speed  $21.11$  mm/s  $\pm$   $7.9$  SD; frequency  $12.9$  /min  $\pm$   $1.8$  SD; n=9). Erythromycin lactobionate ( $10^{-6}$  M), shown to trigger migrating motor complexes (2), initiated irregular rings of circular muscle contractions, which slowly propagated aborally at speed of  $1.9 \pm 0.4$  mm/s (SD; n=7). Within the slowly propagating area of circular muscle contractions, the short rings of muscle contraction propagated at the speed of the myogenic contractions. Hexamethonium  $100\mu$ M blocked the slowly propagating contractions (n=3). Conclusions: Thus the isolated rabbit small intestine can be used to investigate the interaction between myogenic, propulsive movements and migrating complexes. References: 1. Hennig et al (1999), J. Physiol., 517, 575-590; 2. Marzio et al (1994) Peptides, 15, 10067-1977.

## POS-TUE-069

## RECEPTORS TO DETECT SHEAR IN THE WALL OF THE COLON

Chen B.N. and Brookes S.J.H.

Human Physiology &amp; Centre for Neuroscience, Flinders University of South Australia. BEDFORD PARK, S.A. 5042.

Extrinsic sensory nerves to gut give rise to gastrointestinal sensations and reflexes. We aim to characterize systematically major classes of gut afferents according to responses and morphology. Biotinamide labeling was combined with extracellular recordings from colonic nerves, in isolated specimens of colon taken from humanely killed guinea pigs. Recordings with all layers present (n=9) revealed a population of sensory axons that could be strongly activated by probing or stroking with light von Frey hairs (0.1 – 10mN, maximum instantaneous firing:  $83 \pm 12$ , n=7) and by isotonic circumferential stretch (10mN – 100mN loads, maximum instantaneous firing:  $59 \pm 12$ , n=7). They therefore correspond to "muscle-mucosal" afferents previously reported. Removal of the muscularis externa (n=11) had no detectable effect on their excitability, suggesting that they have transductive endings in the submucosa or mucosa. These primary afferents fired in synchrony with spontaneous contractions of the muscularis mucosa and of the muscularis externa (when present). They did not respond to locally applied capsaicin (n=5) and their action potentials had larger amplitude ( $283 \pm 123$  vs  $133 \pm 48$ ,  $P < 0.05$ , n=5) and trended towards shorter half duration ( $440 \pm 120$  vs  $1250 \pm 1005$ , n=5, NS) than capsaicin-sensitive units in the same preparations. Their mechanosensitivity was not blocked by application of low calcium solution (n=5), suggesting that they are intrinsically mechanosensitive rather than relying on release of mediators from other cells. Dye filling revealed a single type of ending that corresponded to their receptive fields. These axons branched extensively in the sub-epithelial plexus, close to the base of the colonic glands. This class of receptor has the ideal characteristics to detect shear stimuli combined with small distension - of the sort probably caused by propulsion of solid faecal pellets.

## POS-TUE-070

## MECHANISM UNDERLYING STRETCH-ACTIVATION OF COLONIC MIGRATING MOTOR COMPLEXES

Zagorodnyuk V.P., Kyloh M. and Spencer N.J.

Department of Human Physiology, Flinders University, South Australia.

It is well known that colonic distension can evoke a premature colonic migrating motor complex (CMMC), but the mechanism underlying this process is unknown. Studies have suggested that distension of the gut wall releases 5-HT from the mucosa that is necessary for the activation of intrinsic nerve endings in the mucosa which then initiate propulsive motor patterns, such as the CMMC. In this study, we investigated whether removal of the mucosa, submucosa and submucosal plexus prevents colonic distension-evoked CMMCs in isolated mouse colon. The entire colon was removed from C57BL/6 mice and a longitudinal incision made along the full length of colon. Results: Graded increases in m/s applied to the mid/distal region of circumferential stretch (30% at 100 colon reliably evoked a premature CMMC in the mid/distal colon and the proximal colon. Complete removal of the mucosa, submucosa and submucosal plexus did not prevent spontaneous or evoked CMMCs, and actually decreased the stretch threshold required to evoke premature CMMCs (control:  $2.1 \pm 0.1$  mm, n=5; mucosa off:  $1.6 \pm 0.04$  mm, n=5,  $P < 0.01$ ), with no effect on the amplitude or duration of stretch-evoked CMMCs (n=5). Real time amperometric recordings confirmed that all dynamic release of 5-HT was prevented in preparations devoid of mucosa, but where stretch reliably evoked CMMCs. Hexamethonium (100µM) abolished spontaneous and stretch-evoked CMMCs (n=5). Conclusions: Neither the release of 5-HT from the mucosa, nor even the presence of the mucosa or submucosal plexus is necessary for the mechanism underlying distension-evoked CMMCs. We suggest that circumferential stretch evokes propagating CMMCs by activating a population of intrinsic sensory neurons that lie in the myenteric plexus and that stimulation of nerve endings in the mucosa is not required for this process.

## POS-TUE-071

## DOWNREGULATION OF CANNABINOID CB1 RECEPTORS IN COLONIC MUCOSA BUT NOT MUSCLE, IN FEMALES WITH SLOW TRANSIT CONSTIPATION

Markus I.<sup>1</sup>, King D.W.<sup>2</sup>, Perera D.S.<sup>2</sup>, Burcher E.<sup>1</sup> and Liu L.<sup>1</sup><sup>1</sup>School of Medical Sciences, University of New South Wales.<sup>2</sup>Colorectal Surgery, St George Hospital, Sydney.

Slow transit constipation (STC) is an example of extreme dysmotility of the human gastrointestinal tract. STC is a disorder of unknown aetiology; it affects mainly women and is characterised by the inability to defecate more than once every 2-3 weeks. Endocannabinoids are important mediators in regulating intestinal function. The cannabinoid CB1 receptors are predominantly localised to myenteric neurons and submucosal neurons where they mediate intestinal motility and water exchange, mainly through an inhibitory action on cholinergic enteric neurons. In this study, we aimed to determine whether CB1 receptor expression is altered in STC. Sigmoid colon segments were obtained from age-matched female patients (aged 23-76 years) undergoing resection for STC (n=12), or for carcinoma (control, n=8). Segments were dissected into smooth muscle and mucosa layers for separate RNA extraction and real time PCR. In STC mucosa (containing submucosal plexus), there was a 25-fold down-regulation of CB1 receptor mRNA ( $P < 0.01$ , Mann Whitney test) compared to control. In contrast, no change was observed in muscle (containing myenteric plexus). The expression of CB2 receptor mRNA was very low in both mucosa and muscle without difference between STC and control. Similar to CB1, mRNA level for synaptophysin, a neuronal marker, was reduced in STC mucosa (6-fold reduction compared to control,  $P < 0.01$ ), but with no differential expression in muscle. These results suggest that secretomotor neuron dysfunction may occur in STC, which is manifested by marked changes in CB1 and synaptophysin gene expression. Thus changes in function may occur in the colonic mucosa, which is currently overlooked in research on pathophysiology of STC.

## POS-TUE-072

## THE EFFECT OF PLANT-DERIVED CHEMICALS ON HISTAMINE EVOKED CONTRACTION OF SMOOTH MUSCLE

Poyton C.N. and Lavidis N.A.

Synaptic Biology Group, School of Biomedical Sciences, Faculty of Science, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia.

The plant-derived chemicals 1,8-cineole, α-pinene, *cis*-3-hexen-1-ol, lavender, linalool and *trans*-2-hexenal are commonly found in many household products and are widely used in complementary medicine. However, the effect of these plant-derived chemicals on histamine evoked contraction of smooth muscle has not been well characterised. **Purpose:** The present study examined the effect of 1,8-cineole, α-pinene, *cis*-3-hexen-1-ol, lavender, linalool and *trans*-2-hexenal on histamine evoked contraction of guinea pig ileum. **Methods:** A guinea pig ileum was mounted in an organ bath and the contraction evoked by histamine in the presence of 1,8-cineole (n=6), α-pinene (n=6), *cis*-3-hexen-1-ol (n=6), lavender (n=6), linalool (n=6), *trans*-2-hexenal (n=6) or no plant-derived chemical (control) (n=12) was recorded. A concentration-response curve was generated by adding increasing concentrations of histamine ranging from  $1 \times 10^{-9}$  M to  $3 \times 10^{-4}$  M. All plant-derived chemicals were examined at a concentration of 0.03% (vol/vol). **Results:** Lavender, linalool and *trans*-2-hexenal induced an extremely significant reduction ( $p < 0.001$ ) in histamine evoked contraction from  $1 \times 10^{-7}$  M to  $3 \times 10^{-4}$  M, 1,8-cineole induced a significant reduction ( $p < 0.05$ ) in histamine evoked contraction from  $3 \times 10^{-7}$  M to  $1 \times 10^{-6}$  M and an extremely significant reduction ( $p < 0.001$ ) in histamine evoked contraction from  $3 \times 10^{-6}$  M to  $3 \times 10^{-4}$  M and α-pinene and *cis*-3-hexen-1-ol did not significantly reduce ( $p > 0.05$ ) histamine evoked contraction. **Conclusion:** Our present findings indicate that lavender, linalool, *trans*-2-hexenal and to a lesser extent 1,8-cineole reduce histamine evoked contraction of smooth muscle.



## POS-TUE-073

## PYY AVAILABILITY IS UNCHANGED IN RAT SMALL INTESTINE DURING A HIGH-FAT DIET

Greathall M.<sup>1,2</sup>, Markus I.<sup>1,2</sup>, Senadheera S.<sup>1,2</sup>, Bertrand R.L.<sup>1</sup>, Liu L.<sup>2</sup> and Bertrand P.P.<sup>1</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences, UNSW.

<sup>2</sup>Department of Pharmacology, School of Medical Sciences, UNSW.

**Purpose:** A high fat diet (HFD) is associated with subtle changes in gastrointestinal (GI) function and in the levels of intestinal hormones secreted from enteroendocrine cells. One such hormone is peptide-tyrosine-tyrosine (PYY) which has a role in central appetite control and in local GI function. How local PYY synthesis is changed in a rat HFD model of obesity is unknown. **Methods:** Rat ileum was taken from control (age-matched, chow-fed, CON: 450-550g; n=20) and high-fat diet rats (HFD: 700-800g; n=28). The mRNA levels of PYY were determined by quantitative RT-PCR and normalised against GAPDH,  $\beta$ -actin and the brush border protein villin. Full-thickness, intact ileum was compared against tissue composed of mucosa-lamina propria. The mRNA level was expressed as fold change =  $2^{-\Delta\Delta Ct}$ . Unpaired, non-parametric data were compared using a Mann-Whitney test ( $P < 0.05$ ). **Results:** Intact ileum showed a pronounced decrease in PYY mRNA in HFD compared to control using GAPDH (CON:  $0.68 \pm 0.32$  n=8; HFD:  $0.10 \pm 0.03$  n=14;  $P < 0.05$ ) and  $\beta$ -actin. However, when mucosa-lamina propria was analysed separately, there was no change in PYY mRNA levels using GAPDH ( $1.49 \pm 0.74$  n=8;  $1.39 \pm 0.38$  n=11) or  $\beta$ -actin. Similarly, when villin was used as a loading control, PYY was unchanged in the intact ileum ( $0.31 \pm 0.14$  n=8;  $0.27 \pm 0.07$  n=15) and in the mucosa-lamina propria. Intact ileum showed reduced villin versus GAPDH in HFD, but no difference in mucosa-only samples. Changes in the non-mucosal compartment were tested using markers of smooth muscle cells. Intact ileum showed no change in either marker in HFD rats using calponin ( $2.00 \pm 0.78$  n=10;  $1.70 \pm 0.62$  n=10) or smoothelin ( $1.45 \pm 0.45$  n=10;  $1.70 \pm 0.44$  n=9). **Conclusions:** Our data shows that PYY mRNA levels are not changed in a rat model of obesity but suggest that other structural changes are taking place in the non-mucosal compartment. Overall, our data predict that PYY availability during a high fat diet is unchanged.

## POS-TUE-074

## NEURONAL ACTIVITY IN DEVELOPING ENTERIC NEURONS

Hao M.M.<sup>1</sup>, Boesmans W.<sup>3</sup>, Van Den Abbeel V.<sup>3</sup>, Bornstein J.C.<sup>2</sup>, Young H.M.<sup>1</sup> and Vanden Berghe P.<sup>3</sup>

<sup>1</sup>Department of Anatomy and Cell Biology, the University of Melbourne, Parkville, Victoria, Australia. <sup>2</sup>Department of Physiology, the University of Melbourne, Parkville, Victoria, Australia. <sup>3</sup>Centre for Gastroenterological Research, Katholieke Universiteit Leuven, Leuven, Belgium.

The enteric nervous system arises from neural crest cells that migrate into the gastrointestinal tract during development. A sub-population of enteric neural crest cells expresses pan-neuronal markers at early stages of development (E10.5 in the mouse). However, when developing enteric neurons become electrically active is unknown. To study this, we used calcium imaging to examine enteric neurons that were dissociated from embryonic gut and then cultured overnight. Sharp increases in intracellular calcium ion concentration ( $[Ca^{2+}]_i$ ) follow action potentials in mature enteric neurons, and can be elicited by electrical field stimulation (EFS). We found that a sub-population of enteric neurons isolated from E11.5 mice responded to EFS (20mA for 2 seconds at 20 Hz) with a peak in  $[Ca^{2+}]_i$  similar to that of adult neurons. The proportion of responding neurons and the amplitude of their responses increased dramatically between E11.5 and E12.5, and further increased in preparations from older embryos. The calcium transients were dependent on voltage-gated sodium channels as they were abolished or diminished in the presence of tetrodotoxin (TTX). Several types of voltage-gated calcium channels were involved, but their roles differed with embryonic age. Furthermore, the majority of neurons from E11.5 embryos responded to DMPP (an agonist of nicotinic cholinergic receptors), and to ATP. Thus, neuronal activity is present very early during the development of the enteric nervous system.

## POS-TUE-075

## AXONAL DAMAGE AND PLASTICITY FOLLOWING INTESTINAL INFLAMMATION

Nurgali K.<sup>1</sup>, Qu Z.<sup>2</sup>, Hunne B.<sup>2</sup>, Thacker M.<sup>2</sup>, Pontell L.<sup>2</sup> and Furness J.B.<sup>2</sup>

<sup>1</sup>Department of Physiology, The University of Melbourne, Parkville, Victoria. <sup>2</sup>Department of Anatomy and Cell Biology, The University of Melbourne, Parkville, Victoria.

The **aim** of this study was to investigate the early stages following the induction of inflammation and mechanisms triggering intracellular changes in the subpopulations of enteric neurons which can lead to long-term pathological changes in neuronal properties. **Methods:** Inflammation was induced by injecting TNBS (30mg/kg in 30% ethanol) into the guinea-pig ileum. Segments of inflamed ileum were examined at 3, 6, 12, 24 hours and 7 days post-TNBS injection or sham operation and compared to control ileum. Inflammation was quantified histologically and immunochemically. Nerve fibre bundles were labeled with anti- $\beta$ Tubulin, anti-GAP-43, anti-tau and anti-phospho-Tau antibodies followed by double-staining with anti-calbindin antibody specific to Dogiel type II (DII) neurons. The number of nerve bundles was visualized and quantified in mid-villi sections. Changes in the electrophysiological properties of enteric neurons were investigated by intracellular recording technique followed by morphological identification of the neuron type. **Results:** This study provides evidence that significant axonal damage occurs at 3-24 hours after induction of inflammation in the guinea-pig ileum. Increase in the number of nerve fibers projecting to the mucosa, possibly due to axonal sprouting, occurs at later stages (day 7 post-TNBS). Neurons projecting to the mucosa that were affected by inflammation include DII neurons from the myenteric plexus which functionally are the intrinsic primary afferent neurons. Significant increases in excitability of DII neurons recorded at 3 (n=18) and 24 hours (n=20) after the induction of inflammation. **Conclusion:** This is the first functional study of the very early changes in enteric neurons following an inflammatory challenge. Intestinal inflammation causes damage to the neuronal processes in the mucosa and rapidly increases neuronal hyperexcitability.

## POS-TUE-076

## HISTOLOGICAL CHANGES IN THE ILEUM AFTER INTESTINAL ISCHEMIA AND REPERFUSION

Pontell L., Rivera L.R., Thacker M. and Furness J.B.  
Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia.

A period of ischemia followed by reperfusion (I/R) of the intestine causes long-term changes in motility. We have recently found degenerative changes in enteric neurons and glia after I/R that could cause motility changes. In the present work, we have investigated changes in muscle and other cell types that could also contribute to functional deficits. **Purpose:** The aim was to provide a detailed analysis of the histological changes that occur in the mouse ileum following I/R. **Methods:** I/R was achieved in the ileum of anaesthetised mice by occluding a branch of the superior mesenteric artery for 1hr. Reperfusion was allowed to occur from 1hr to 7days. Ileum samples (from 20 animals) were taken from within the occluded area and from a non-occluded area proximal to the occlusion. The samples were prepared for haematoxylin and eosin histology. **Results:** At 1hr the epithelium was broken and sloughing off into the lumen; at 3hr the villi appeared flattened but the epithelium had regrown. Numerous intra-epithelial inclusions were present. At 24hr cells of the longitudinal muscle layer were separated and contained vacuoles. By 48hrs they were rounded and pale staining, but still contained normal nuclei. By 7 days the morphology of the muscle appeared normal. There were no changes in the epithelium or muscle in the non-occluded region. In comparison, neuronal and glial cell damage was observed in both regions. **Conclusion:** The motility disorders that are observed after I/R probably involve damage to both neurons and muscle. The muscle cells appear to de-differentiate to a synthetic phenotype and then return to a contractile phenotype. This needs to be confirmed by functional studies.

## POS-TUE-077

**IDENTIFICATION OF A NOVEL NEURO-NEURONAL PATHWAY THAT UNDERLIES PERISTALSIS AND FECAL PELLET PROPULSION IN GUINEA-PIG DISTAL COLON**

Gregory S. and Spencer N.  
Flinders University South Australia.

The mechanism by which distension of the colon activates peristalsis and the propulsion of colonic contents is poorly understood. In this study, we used video imaging and spatio-temporal mapping techniques to investigate the neuronal mechanisms underlying peristalsis in isolated guinea-pig distal colon. In contrast to previous studies, we found that hexamethonium (100µM–1mM) or mecamlamine (20µM) never abolished peristalsis or fecal pellet propulsion. In hexamethonium or mecamlamine, further addition of PPADS (10µM), ondansetron (1µM) and SR 142-801 (300nM) had no inhibitory effect on the propagation velocity of fecal pellets. During the initiation of peristalsis, the intraluminal propulsive force applied to an inserted fecal pellet was significantly reduced by hexamethonium 100µM (control of  $8.9 \pm 1.1$  to  $4.1 \pm 0.25$  mN in hexamethonium;  $n=5$ ;  $P<0.05$ ). Although, the rate-of-rise of tension developed by the non-nicotinic pathway on the fecal pellet was not significantly different in the presence of hexamethonium (control:  $0.49 \pm 0.2$  g.sec<sup>-1</sup> to  $0.42 \pm 0.1$  g.sec<sup>-1</sup> in hexamethonium;  $n=5$ ;  $P>0.05$ ). The reduction in propulsive force in hexamethonium was not associated with a reduction in propagation velocity of fecal pellets. When the distal colon was removed from guinea-pigs (with endogenous fecal pellets present) and placed immediately into hexamethonium (100µM), peristalsis and endogenous fecal pellet propulsion along the colon was abolished, so that all endogenous pellet movement ceased. However, in these same preparations whilst in hexamethonium, local acute stimulation of the colon, such as insertion of exogenous fecal pellets triggered peristalsis and induced propulsion of fecal pellets. Taken together, nicotinic transmission plays an essential role in the natural propulsion and expulsion of endogenous fecal pellets. However, a novel non-nicotinic pathway, can also be activated, by local acute stimulation, which can generate peristalsis and fecal pellet propulsion at normal propagation velocities, but does not require purinergic (P2), serotonergic (5-HT3) or tachykinergic (NK3) receptors. The non-nicotinic pathway appears to require acute stimulation for its activation, and is not sufficiently activated by maintained distension by multiple endogenous pellets.

## POS-TUE-079

**ELECTROPHYSIOLOGICAL CHANGES IN ENTERIC NEURONS FOLLOWING ISCHEMIA/REPERFUSION INJURY**

Rivera L.R., Nguyen T.V., Thacker M., Pontell L. and Furness J.B.  
Department of Anatomy and Cell Biology, University of Melbourne, Australia.

Damage following ischemia and reperfusion (I/R) injury is common in the intestine, and can be caused during abdominal surgery, in several disease states and following intestinal transplantation. I/R results in changes in motility, implying that properties of motility-controlling neurons are altered. Previous investigations in this laboratory have shown that a brief period of ischemia, followed by reperfusion, causes sustained changes in specific subtypes of enteric neurons. Purpose: There have been no reported electrophysiological investigations in enteric neurons following ischemia. Therefore, this study was designed to determine whether there is a functional correlate of the structural changes that were observed in neurons following I/R. Methods: A branch of the superior mesenteric artery of anaesthetised guinea-pigs was occluded for one hour. Tissues from the occluded and non-occluded regions (5 cm oral from the occlusion site) were taken for in vitro investigation 24 hours later. Myenteric neurons were investigated using intracellular microelectrodes and the neurons were filled with marker dyes from the recording electrode to determine their morphologies. Results: The majority of the neurons that have been electrophysiologically classified as AH neurons ( $n=11$ ) and a small proportion of S neurons ( $n=4$ ) became hyperexcitable (average of 15 spikes in response to a depolarisation step of 0.3nA, 500ms). These neurons also exhibited anodal break action potentials (after hyperpolarisation of -5mV, 100ms from the resting membrane potential), and some of these neurons showed spontaneous action potentials in both the occluded and non-occluded regions. Some S neurons appeared to be unaffected. Conclusion: We conclude that I/R injury causes hyper-excitability of particular subtypes of enteric neurons. These changes may contribute to the dysmotility that is observed after intestinal I/R.

## POS-TUE-078

**NICOTINIC PATHWAYS AND THEIR CONTROL OVER CYCLICAL MOTOR PATTERNS UNDERLYING COLONIC PROPULSION**

Costa M., Spencer N. and Brookes S.J.H.  
Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, South Australia.

Propulsion of pellets in the colon involves both acute distension activation of enteric circuits and cyclic motor complexes (1). Nicotinic transmission may be not essential for the propulsion of single pellets (2). Purpose: To investigate the role of nicotinic transmission in distension evoked cyclic motor complexes and pellet propulsion in the same preparation. Methods: segments of distal colon from 5 adult guinea-pigs killed humanely, were placed in organ bath with Krebs at 37°C. Video spatio-temporal maps of changes in length and diameter were constructed (3) during short and long fixed balloon distensions and during interrupted and uninterrupted artificial pellet propulsion. Results: short balloon distensions (20-30s) elicited oral contraction of the circular muscle and longitudinal shortening over the entire segment, which were reduced but not abolished by Hexamethonium (100µM). Distensions of 15-20min elicited similar muscle contractions in cycles at frequency of  $0.27 \pm 0.03$  cycles/min SEM). Hexamethonium reduced the amplitude of cyclic contractions but did not affect their frequency ( $0.34 \pm 0.15$  cycles/min SEM;  $n=5$ ). These cyclic contractions exerted of propulsive force on held pellets, which was significantly reduced by hexamethonium ( $7.31 \pm 1.18$ g to  $2.31 \pm 0.80$ g SEM,  $n=5$ ). However, after being held fixed, pellets cut free to move were still propelled in hexamethonium at a similar speed as in controls ( $2.73 \pm 1.37$  vs  $2.56 \pm 1.28$ mm/s SEM;  $n=5$ ). Conclusions: propulsion of single pellets in the guinea-pig distal colon occurs independently from cyclic motor activity and requires minimal propulsive force that does not involve nicotinic enteric pathways. References: 1. Costa and Furness (1976). Naunyn Schmied Arch Pharmacol. 16, 294:47-60. 2. Gregory and Spencer, J Physiol (submitted). 3. Hennig et al (1999), J. Physiol., 517, 575-590.

## POS-TUE-080

**TRANSABDOMINAL ELECTRICAL STIMULATION INCREASES COLONIC ACTIVITY IN CHILDREN WITH CHRONIC CONSTIPATION**

Southwell B.R.<sup>1,3</sup>, Chase J.<sup>1</sup>, Gibb S.<sup>2</sup>, Clarke M.M.<sup>1</sup>, Ismail K.A.<sup>3</sup>, King S.K.<sup>1,3</sup>, Reilly D.<sup>3</sup>, Chow C.S.<sup>3</sup>, Catto-Smith T.G.<sup>2</sup> and Hutson J.M.<sup>1,2,3</sup>  
<sup>1</sup>Murdoch Childrens Research Institute. <sup>2</sup>Royal Childrens Hospital, Melbourne. <sup>3</sup>Department of Paediatrics, University of Melbourne.

Background: All neurobiologists recognise that electricity activates nerves. Physiotherapists specialise in applying transcutaneous electrical stimulation (TES). TES used to treat bladder overactivity produces diarrhoea, suggesting that TES speeds up bowel transit. In a pilot study, TES increased defecation in 5/8 children with chronic constipation. Chronic slow transit constipation (STC) is resistant to medical management and is marked by soft stools that accumulate in the proximal colon and soiling. Aim: Determine if TES improves colonic function in pediatric STC. Methods: 46 children (8-18 yr) with STC (confirmed by radionuclear transit study) were randomly assigned to active(A) or sham(S) stimulation (20 min, 12 sessions, 3/wk). Two (4 x 4 cm) adhesive electrodes were placed paraspinally and 2 on the abdomen near the belly button (T9-L2). Stimulation was just below sensory threshold (<40mA, carrier-frequency 4kHz, beat frequency 80-150Hz). Daily diaries recorded defecation and soiling for 1mth baseline, 1 mth during and 2mths after stimulation. Quality of life (PedsQL) and colonic transit (scintigraphy) were compared before and 2 mths after treatment. 5 children had 24-hr colonic manometry before and 2 mths after active stimulation. 11 children also received daily stimulation for 2 mths, 12 mths after completing the RCT. Results: 42 children (8-18 yr, 20 male,) completed the RCT. Active stimulation resulted in faster colonic transit times (Geometric centre at 48 hrs, mean±SEM: A4.28±0.03 vs S3.22±0.05,  $p=0.007$ ), less abdominal pain ( $0.60 \pm 0.16$  vs  $1.8 \pm 0.47$  days/wk,  $p=0.0002$ ), less soiling ( $1.92 \pm 0.43$  vs  $2.63 \pm 0.56$  episodes/wk,  $p=0.002$ ), and improved self-perceived physical quality of life ( $83.9 \pm 3.0$  vs  $76.7 \pm 3.5$ ,  $p=0.01$ , A). There was no change in defecation in children stimulated 3 times/wk, but with stimulation daily, defecation increased into the normal range ( $2.5 \pm 2.1$  to  $6.7 \pm 4.4$  episodes/wk,  $p=0.008$ ). Conclusion: Colonic transit, soiling, QOL and defecation improved in children given TES. The effect is dose-dependent. TES could provide a treatment for chronic constipation.

## POS-TUE-081

### INVESTIGATING THE CONTRIBUTION OF ENTEROCHROMAFFIN CELL DISTRIBUTION TO LOCAL 5-HT AVAILABILITY

Tan K.L.<sup>1</sup>, Bertrand R.L.<sup>1</sup>, Senadheera S.<sup>1,2</sup>, Tanoto A.<sup>1</sup>, Liu L.<sup>2</sup> and Bertrand P.P.<sup>1</sup>

<sup>1</sup>Department of Physiology. <sup>2</sup>Department of Pharmacology.

Local availability of 5-HT at the mucosal surface of the intestine is related to the amount of 5-HT released by enterochromaffin (EC) cells, the number of EC cells in the area and the subsequent re-uptake of 5-HT. Our previous data showed that the local availability of 5-HT increases dramatically with inflammation or during obesity, and is coupled with increased numbers of EC cells. We aimed to determine the relationship between the concentration of 5-HT at the mucosal surface and the number of EC cells within small regions of colon. Distal colon was taken from control (n=9) and high-fat diet rats (n=11). 5-HT concentrations near the mucosal surface were measured electrochemically during steady state (SS) and peak evoked (compression) release. Eight spots from each preparation were tested with 5-8 repetitions (n=6) and showed overall peak 5-HT of 16.1 $\mu$ M and SS 5-HT of 4.7 $\mu$ M. Individual spots showed a high (27%) or low variability (73%) in 5-HT levels which were well correlated between peak and SS measurements. EC cell numbers were determined by counting immunohistochemically labelled 5-HT cells in cross-sections. The variability over 1,973 EC cells in 2542 crypts (n=13) also showed high (n=5) and low variability (n=7). Our data suggests that the variability in the electrochemical recordings between spots is of the same order of magnitude as seen in the EC cells counts. However, the electrochemical recordings also varied widely within single spots. This suggests that the function of the EC cells also varies or that the distribution of EC cells is important on a smaller scale than can be assessed electrochemically.

## POS-TUE-082

### NEURONAL DEATH AND GLIAL CELL DAMAGE OCCURS IN MOUSE ILEUM FOLLOWING INTESTINAL ISCHEMIA AND REPERFUSION

Thacker M.<sup>1</sup>, Rivera L.R.<sup>1</sup>, Pontell L.<sup>1</sup>, Castelucci P.<sup>2</sup>, Sharkey K.A.<sup>3</sup> and Furness J.B.<sup>1</sup>

<sup>1</sup>Department of Anatomy and Cell Biology, University of Melbourne, Australia. <sup>2</sup>Department of Anatomy, University of Sao Paulo, Brazil.

<sup>3</sup>Department of Physiology and Biophysics, University of Calgary, Canada.

Neuronal changes following ischemia reperfusion (I/R), in the guinea pig ileum, have previously been documented by this laboratory. However, the extent of neuronal damage/death has not been determined. Interestingly, the effects of intestinal I/R on enteric glia remains unexplored despite the knowledge that glia contribute to neuronal maintenance and survival. Purpose: The aim of this study was to assess damage to enteric glia and quantify the neuronal loss following intestinal ischemia reperfusion (I/R) in mouse ileum. Method: A branch of the superior mesenteric artery in anaesthetised C57/Blk6 or S100-GFP mice was occluded for 1 hour and the animal was allowed to recover for a period of 1 hour to seven days. Tissue was taken from both occluded and non-occluded regions of the ileum. Immunohistochemical methods were used for the investigation of specific neuronal and glial cell markers, including indicators of cell death (TUNEL) and protein nitrotyrosylation (3-NT). Results: There were significantly more neurons (p<0.05) and glial cells (p<0.01) containing 3-NT in the occluded regions from 6-24 hours following I/R compared to non-occluded and control regions (no 3-NT). A small percentage of neurons displayed TUNEL immunoreactivity 6 hours after I/R. Glial degradation was visualized using the S100-GFP mice and glial fibrillary acidic protein (GFAP) immunohistochemistry. GFAP re-organisation and ablation of the S100-GFP protein was evident 3 hours following I/R. Conclusion: The data suggests that glial cell damage precedes neuronal loss; thus the glial damage may contribute to neuronal degradation.

## POS-TUE-083

### SOMATO-ADRENAL REFLEX AND UPPER CERVICAL SPINAL CORD COMPRESSION – A PILOT STUDY

Budgell B.S.<sup>1</sup> and Bolton P.S.<sup>2</sup>

<sup>1</sup>Canadian Memorial College of Chiropractic, Toronto, Canada.

<sup>2</sup>School of Biomedical Sciences & Pharmacy, University of Newcastle, Australia.

**Purpose:** The aim of this study was to test whether applying transient (range 10-60 min) compression to the upper cervical spinal cord modulates somatic evoked reflex activity in the adrenal nerve. **Methods:** Experiments were performed on spontaneously breathing adult Wistar rats (n=5; 380-430g) initially anaesthetized with urethane (1.3g/kg i.p.) and supplemented (i.v.) to maintain absence of withdrawal and palpebral reflexes. Venous and arterial canulas provided fluids and a record of arterial blood pressure. Averaged adrenal nerve activity was recorded in response to electrical stimulation (1Hz, 5 X 0.5ms square wave pulses) of the ipsilateral sciatic nerve at  $\geq 1.5X$  threshold (T) for muscle twitch, while static compression was applied using a probe (2.3 X 2.8 mm) placed on the dorsal surface of the exposed, dura intact, upper cervical spinal cord. **Results:** High intensity ( $\geq 15T$ ) stimuli evoked a reflex response (onset latency range 50-100ms; duration ~120ms) in each rat's stimulus-triggered averaged (n=500) adrenal nerve recordings. Applied pressure ranging (1.13-3.92g) from that sufficient to compress the dura so it just contacted the dorsal surface of the cord to that necessary to occlude the vessels on the dorsal surface of the cord, induced a reduction (range 12-35%) in the amplitude of the somatic evoked adrenal nerve response. When tested up to 60 min after removing the probe, the somatic evoked responses were present but remained reduced in amplitude. **Conclusion:** In the anaesthetized rat, static transient (< 60 min) compression of the upper cervical spinal cord can reduce somatic afferent induced activity in the adrenal nerve and it remains reduced for more than an hour after compression has been removed.

## POS-TUE-084

### NEUROVASCULAR TRANSMISSION IS IMPAIRED IN ARTERIES SUPPLYING SKIN OF DIABETIC RATS

Johansen N.J., Tripovic D. and Brock J.A.

Prince of Wales Medical Research Institute, University of NSW, Sydney, NSW 2031.

Abnormal neural control of skin blood flow is implicated in pathogenesis of diabetic foot. The mechanisms whereby diabetes affects the neural control of skin vasculature are not understood but it is believed to cause perivascular nerve loss. Here we investigated the effects of diabetes on sympathetic neurovascular transmission in two arteries supplying skin (tail artery, planter metatarsal arteries) and compared them with those in mesenteric arteries. Rats were made diabetic with streptozotocin (60 mg/kg, i.p.). Twelve weeks after the induction of diabetes, artery segments were removed under terminal anaesthesia with pentobarbitone (100 mg/kg i.p.) and mounted isometrically. Comparisons were made with arteries from vehicle-treated rats. In both tail artery (n=5) and planter metatarsal arteries (n=6), diabetes reduced nerve-evoked constrictions ( $P < 0.01$  for both comparisons). In contrast, nerve-evoked constrictions of mesenteric arteries were unaffected by diabetes (n=6,  $P=0.33$ ). In both cutaneous arteries, diabetes did not affect the sensitivity to  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor agonists (phenylephrine and clonidine respectively;  $P > 0.65$  for all comparisons). Furthermore, diabetes did not change the leftward shift in the concentration response curve for phenylephrine produced by blockade of the neuronal noradrenaline transporter with desmethylinipramine (30 nM;  $P > 0.1$  for both comparisons). As sympathetic denervation reduces neuronal uptake of phenylephrine and thereby increases vascular sensitivity to this agent [1], these findings suggest reduced neural activation of these vessels cannot be attributed to nerve loss. Instead, the findings suggest neurovascular transmission is impaired in these vessels. In conclusion, diabetes impairs neurovascular transmission in arteries supplying skin and this change precedes nerve loss (if it occurs). 1. Tripovic et al. (2009) *Br J Pharmacol.* In press.



## POS-TUE-085

**DIFFERENTIAL CONTRIBUTION OF NEURONAL NITRIC OXIDE TO CEREBRAL ARTERIES OF THE RAT**

Chua A.H.C. and Hill C.E.

The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200 Australia.

Nitric oxide (NO) released from parasympathetic nerves causes vasodilatation of large cerebral arteries and increases cerebral blood flow in vivo<sup>1</sup>. However, the relative importance of nitrgic nerves to control of cerebral vessels of different sizes has not been determined. The present study therefore aimed to compare nerve-mediated responses of pressurized vertebral arteries (351 ± 10µm maximal diameter) with those of caudal cerebellar arteries (193 ± 2µm maximal diameter) of the rat. Immunohistochemistry demonstrated a dense plexus of neuronal NO synthase-containing nerves surrounding the basilar and vertebral arteries, while the caudal cerebellar arteries showed sparse nitrgic innervation and nitrgic nerves were absent from smaller pial arterioles. Electrical field stimulation (10Hz; 5s trains) of both vessels elicited vasodilatation. In order to eliminate the influence of sensory nerves, experiments were conducted in the presence of capsaicin (10µM), which significantly reduced tone in the vertebral, but not in the caudal cerebellar artery. In the vertebral artery, effects of capsaicin in reducing nerve-mediated responses could be attributed to the loss of tone, while in the caudal cerebellar artery, nerve-mediated vasodilation was significantly reduced in amplitude. Inhibition of NO with L-NAME (10µM) significantly increased tone relative to control in the vertebral artery and eliminated nerve-mediated vasodilation (n=4) but not in the caudal cerebellar artery (n=3). We conclude that nitrgic nerves are the main contributor to vasodilator responses of the vertebral artery, while sensory nerves play a role in the smaller caudal cerebellar artery, along with other NO-independent vasodilatory neurotransmitters. <sup>1</sup>Toda N, Ayajiki K, Okamura T (2009) Cerebral blood flow regulation by nitric oxide: recent advances. *Pharmacol Rev* 61:62-97.

## POS-TUE-087

**ASSYMETRICAL SOMATOSYPATHETIC RESPONSES TO RIGHT AND LEFT SCIATIC NERVE STIMULATION**Korim W.S.<sup>1,2</sup>, McMullan S.<sup>1</sup>, Cravo S.L.<sup>2,3</sup> and Pilowsky P.<sup>1</sup><sup>1</sup>Macquarie University - Australia. <sup>2</sup>Universidade de Sao Paulo - Brazil.<sup>3</sup>Universidade Federal de Sao Paulo Escola Paulista de Medicina - Brazil.

We have previously shown that different patterns of hindlimb bloodflow can be evoked by left versus right sciatic nerve stimulation (L- and R-SN respectively). The aim of this study was to compare changes in left lumbar sympathetic nerve activity (LSNA) evoked by electrical stimulation of the L- and R-SN, and to investigate the central pathways responsible. In urethane anesthetized (1.3 g/kg, i.p.), vagotomized, paralyzed (pancuronium bromide 0.4 mg i.v.) and artificially ventilated Sprague-Dawley rats (N = 9) averages of LSNA responses to L- and R-SN stimulation (0.5 Hz, 0.6 ms, 1 mA) revealed two excitatory peaks (126 ms & 242 ms). Responses to L-SN stimulation were characterized by an inhibitory phase that preceded the first excitatory peak (onset 54 ms), which was absent when the R-SN was stimulated. Microinjection of muscimol (6 mM, N = 6) or kynurenic acid (50 mM, n = 6) into the right rostral ventrolateral medulla (R-RVLM) abolished the excitatory response to L-SN stimulation, leaving the inhibitory phase intact. L-RVLM Muscimol abolished the excitatory response to R-SN stimulation without affecting the response to L-SN stimulation. Cervical spinal cord transection abolished the inhibitory phase evoked by L-SN stimulation. These data suggest that somatic nerve stimulation evokes bilateral sympathoexcitation, which is mediated by glutamatergic transmission in the RVLM, and simultaneous unilateral sympathoinhibition ipsilateral to the sensory nerve. Such sympathoinhibition, generated by unknown supraspinal structures, has not been previously described. The physiological role of this mechanism may be related to the differential control of regional bloodflow in response to nociceptive stimulation and high states of arousal.

## POS-TUE-086

**MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY BY SINUSOIDAL GALVANIC VESTIBULAR STIMULATION IS LOWER WHEN DELIVERED AT THE CARDIAC FREQUENCY**

James C. and Macefield V.G.

School of Medicine, University of Western Sydney.

**Purpose:** Muscle sympathetic nerve activity (MSNA) is normally entrained by the arterial baroreceptors to occur as bursts phase-locked to the cardiac cycle. However, we have previously demonstrated that selective stimulation of vestibular inputs, via sinusoidal galvanic vestibular stimulation (sGVS), can modulate MSNA (Bent et al., 2006) and recently showed that the modulation is weakest at 0.8 Hz and greatest at 0.2 Hz (Grewal et al., 2009). Here we test the hypothesis that frequencies of sGVS delivered at the subject's cardiac frequency causes less modulation than frequencies delivered on either side of the cardiac frequency. **Methods:** MSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 7 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 200 cycles) was applied to the mastoid processes at the cardiac frequency and at 0.1, 0.2, 0.3 and 0.6 Hz above and below this frequency. **Results:** Cross-correlation analysis revealed a cyclic modulation of MSNA at all frequencies, with a clear dip at the cardiac frequency (47.9±3.8%); by contrast, modulation was 57.9±3.6% when delivered 0.1 Hz lower, and 55.1±3.5% when delivered 0.1 Hz higher, than the cardiac frequency. **Conclusions:** We conclude that vestibular inputs compete with baroreceptor inputs operating at the cardiac rhythm, with vestibular modulation of MSNA being lowest when this competition with the baroreceptors is highest. Bent LR, Bolton PS & Macefield VG, Modulation of muscle sympathetic bursts by sinusoidal galvanic vestibular stimulation in human subjects. *Exp Brain Res* 2006 174: 701-711 Grewal T, James C & Macefield VG, Frequency-dependent modulation of muscle sympathetic nerve activity by sinusoidal galvanic vestibular stimulation in human subjects. *Exp Brain Res* 2009 197: 379-386.

## POS-TUE-088

**INTRATHECAL OREXIN A INCREASES SYMPATHETIC OUTFLOW AND RESPIRATORY DRIVE AND MODULATE PHYSIOLOGICAL REFLEXES**

Shahid I.Z. and Pilowsky P.M.

Australian School of Advanced Medicine, Macquarie University, Level 1 Dow Corning Building, 3 Innovation Rd, North Ryde, NSW 2109, Australia.

Orexin A and orexin B, two hypothalamic peptides, are important signalling molecules in feeding and sleep/wakefulness. Orexin containing neurons in the lateral hypothalamus project to numerous areas of the brain and spinal cord including the intermediolateral cell column that contain sympathetic preganglionic neurons. This study was undertaken to determine if orexin A modulates sympathetic output. Experiments were conducted on anesthetized, vagotomized and artificially ventilated Sprague-Dawley rats (n = 16). Intrathecal injections of orexin A caused dose-dependent hypertension, tachycardia and sympathoexcitation. The maximum effect was found at 10 nmol with increases in MAP, HR and sSNA of 30 ± 7 mmHg, 37 ± 6 bpm and 84 ± 18 % respectively. Orexin A also increased phrenic nerve amplitude by 69 ± 11 %. The effects of intrathecal orexin A (10 nmol) on baroreflex, chemoreflex and somatosympathetic reflex were also investigated. Orexin A caused no significant change in baroreflex. It also potentiated the pressor response to stimulation of chemoreflex with 100% nitrogen without significant change in splanchnic sympathetic nerve activity. Orexin A significantly reduced the 2nd peak of somatosympathetic reflex but the 1st peak was unaffected. These findings demonstrate that i) orexin A causes sympathetically mediated increase in MAP and HR and increases inspiratory drive and ii) differentially modulates the reflex activity.

## POS-TUE-089

**NON-L-TYPE CALCIUM CHANNELS CONTRIBUTE TO CEREBROVASCULAR FUNCTION**Kuo I.Y.<sup>1</sup>, Seymour V.S.<sup>1</sup>, Sandow S.L.<sup>2</sup>, Ellis A.<sup>1</sup> and Hill C.E.<sup>1</sup><sup>1</sup>Neuroscience Program, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200 Australia.<sup>2</sup>Department of Pharmacology, School of Medical Sciences, University of New South Wales, Kensington, NSW, 2052, Australia.

Antagonists of L-type voltage dependent calcium channels (VDCCs) are widely used in the treatment of vasospasm following subarachnoid haemorrhage, however their effectiveness in improving patient outcome is questionable. Our aim was to determine whether non-L-type calcium currents contribute to cerebrovascular function, and to determine the selectivity of T-type VDCC antagonists. Quantitative PCR, immunohistochemistry and immunoelectronmicroscopy were used to define the subtypes and location of voltage-dependent calcium channels expressed in cerebral arteries. Patch clamp electrophysiology, pressure myography and pharmacology were used to classify calcium currents in isolated cerebrovascular SMCs and responses of cerebral arteries. MessengerRNA and protein for L- (Ca<sub>v</sub>1.2) and T-type channels (Ca<sub>v</sub>3.1, Ca<sub>v</sub>3.2) were detected in cerebral arteries. In isolated SMCs, a high voltage-activated calcium current with L-type VDCC kinetics and sensitivity to the dihydropyridines, nifedipine and nimodipine, comprised 75% of the current, while the residual current had kinetics typical of T-type currents. Both the dihydropyridine-sensitive and insensitive components could be blocked by T-type blockers, but only the sensitive component was blocked by diltiazem. This component was larger in SMCs from smaller arteries. In large pressurised arteries, voltage-dependent constriction was abolished by L-type antagonists, while in smaller arteries, this required both L- and T-type antagonists. However, considerable overlap in the action of these antagonists was found. We suggest that a heterogeneous population of L-type and high voltage-activated T-type VDCCs contribute to vascular tone in resistance sized cerebral arteries, providing a novel therapeutic target for therapy-resistant vasospasm.

## POS-TUE-090

**CARDIAC BAROREFLEX DELAY: HOW IS IT REDUCED BY CLONIDINE?**McAllen R.M.<sup>1,2</sup>, Toader E.<sup>1</sup>, Cividjian A.<sup>1</sup>, Wesseling K.<sup>3</sup>, Karemaker J.<sup>3</sup> and Quintin L.<sup>1</sup><sup>1</sup>University of Lyon-1 France. <sup>2</sup>Howard Florey Institute, Australia.<sup>3</sup>Academic Medical Center, University of Amsterdam, Netherlands.

The delay between spontaneous rises in blood pressure and baroreflex bradycardia increases when vagal tone is low. Vagal tone is high after clonidine, and we investigated its action on baroreflex delay in humans and rats. In 8 male volunteers, baroreflex delay was assessed by both sequence and cross correlation methods before and after clonidine (6 µg/kg orally). Clonidine lowered heart rate and significantly reduced baroreflex delay ( $P < 0.05$ , both methods), increasing the proportion of sequences showing a delay of 0 vs.1 beat. In anaesthetised rats (urethane, 1.4 g/kg. i.v.), we assessed baroreflex delay from the inverse correlation between heart rate and systolic pressure during inflation of an aortic balloon catheter. Clonidine (100 µg/kg i.v.) lowered blood pressure and heart rate and reduced baroreflex delay ( $n=5$ ,  $p<0.05$ ). Eight cardiac vagal motoneurons, identified electrophysiologically by antidromic activation, showed ongoing activity linked to the arterial pulse; but clonidine caused no shift in its latency from the pulse wave. We then measured heart rate responses to brief supramaximal stimuli to the cervical vagus, and found that timing was critical. Efferent stimuli synchronous with the R-wave slowed the heart maximally after 1 beat, which became 0 beat after clonidine ( $n=5$ ,  $p<0.05$ ). When stimulus timing was varied, and cycle lengthening plotted vs. time after stimulus, clonidine changed neither the latency nor the time course of the cardiac response ( $n=5$  rats,  $p=0.47$ ). In conclusion, central reflex processing time is unchanged by clonidine, but the longer cardiac period allows naturally timed CVM volleys to arrive in time to slow an earlier heartbeat.

## POS-TUE-091

**SYNERGY BETWEEN  $\alpha$ 1-ADRENOCEPTOR SUBTYPES AND WITH P2X RECEPTORS IN NERVE-INDUCED RESPONSES OF MOUSE MESENTERIC ARTERIES REVEALED BY DOUBLE AND TRIPLE KNOCKOUT STRATEGIES**Methven L.<sup>1</sup>, Simpson P.C.<sup>2</sup>, Daly C.J.<sup>1</sup> and McGrath J.C.<sup>1</sup><sup>1</sup>Integrative and Systems Biology, University of Glasgow, Scotland.<sup>2</sup>Department of Medicine, University of California, San Francisco, CA, USA.

It is not known whether sympathetically-mediated constriction of resistance arteries involves all 3  $\alpha$ 1-adrenoceptor (AR) subtypes because of the poor selectivity of antagonist drugs and the complexity of identifying the individual contributions of different receptors. We employed double and triple knockouts of the  $\alpha$ 1-AR to analyse responses to perivascular stimulation of isolated mouse first order mesenteric arteries using wire myography. In wild type arteries ( $n=6$ ), antagonists of either  $\alpha$ 1A-ARs or  $\alpha$ 1D-ARs produced  $>90\%$  blockade of constriction to low frequencies in the physiological range, suggesting these receptor subtypes act synergistically. The results in arteries with only  $\alpha$ 1A-ARs ( $n=6$ ) or  $\alpha$ 1D-ARs ( $n=5$ ) confirmed these observations. After knockout of all 3  $\alpha$ 1-ARs ( $n=5$ ), the small residual constriction was blocked by  $\alpha, \beta$  methylene ATP, indicating it was mediated by P2X receptors. Analysis of knockouts with either  $\alpha$ 1A-ARs or  $\alpha$ 1D-ARs using antagonists revealed synergism between P2X receptors and  $\alpha$ 1A-AR but not between P2X receptors and  $\alpha$ 1D-AR. These data imply powerful synergism between noradrenergic and purinergic responses involving  $\alpha$ 1A-adrenoceptors, pointing to multiple post-receptor signalling interactions. Supported by British Heart Foundation (PG/05/140/20094 and FS/04/035).

## POS-TUE-092

**HEMORRHAGIC STROKE LESION AND THE ALTERATION OF CIRCADIAN BLOOD PRESSURE PATTERN**Nakase T.<sup>1</sup>, Yoshioka S.<sup>1</sup>, Nagata K.<sup>2</sup> and Suzuki A.<sup>1</sup><sup>1</sup>Dept. of Stroke Science, Research Institute for Brain & Blood Vessels-Akita. <sup>2</sup>Dept. of Neurology, Research Institute for Brain & Blood Vessels -Akita.

**Objective:** Importance of domestic blood pressure values and 24 hour ambulatory blood pressure monitoring (24hABPM) has been discussed in the context of risk factor of cerebrovascular diseases. In this study, we evaluated the effect of stroke lesions for the alteration of circadian blood pressure pattern. **Methods:** Hemorrhagic stroke patients admitted to the hospital within 24 hr after the onset were enrolled in this study ( $n=34$ :  $61.6 \pm 10.6$  years-old). 24hABPM was performed every 30 min starting from 1 pm on admission and in following 3 weeks. All patients were classified into dipper and non-dipper types based on the ratio of average daytime and nighttime BP. Urine level of vanillylmandelic acid (VMA) was measured on admission and in following 3 weeks. The hematoma size was calculated based on the findings of brain computed tomography on admission. Lesions were classified into pons, left and right thalamus and left and right putamen ( $n=2, 6, 10, 7$  and  $9$ , respectively). **Results:** There was no significant correlation between the hematoma size and the measurement of blood pressure at the emergency room. However, size of the hematoma was significantly larger in the non-dipper type compared to that in the dipper type observed in 24hABPM in 3 weeks after the onset ( $p=0.016$ ). VMA was significantly decreased in the patients of non-dipper type both on admission and in following 3 weeks ( $p=0.015$ ). All right thalamic lesions showed dipper type in following 3 weeks. **Conclusions:** The size of lesion rather than the blood pressure at the onset can be a predictor of the prognosis of circadian blood pressure pattern. Moreover, the right thalamic hemorrhage may not affect the circadian blood pressure pattern.

## POS-TUE-093

**DORSOMEDIAL HYPOTHALAMUS AND MEDULLARY RAPHE MEDIATE RESPIRATORY AROUSAL RESPONSES IN RATS**Beig M.I.<sup>1</sup>, Xavier C.H.<sup>2</sup>, Fontes M.A.P.<sup>2</sup> and Nalivaiko E.<sup>1</sup><sup>1</sup>University of Newcastle, Callaghan NSW 2308 Australia. <sup>2</sup>Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil.

Alerting sensory stimuli elicit stereotyped tachypnoeic responses in conscious rats. We aimed to reveal which brain areas are involved in the generation of these responses. In adult male Wistar rats instrumented for telemetric ECG transmitters, respiration was recorded using whole body plethysmography. On different days, microinjection of either muscimol or vehicle was made into the dorsomedial hypothalamus (DMH, Group 1, n=6) or into the medullary raphe (MR, Group 2, n=6). Alerting stimuli were presented in the following sequence: tap (acoustic); sudden side move (proprioceptor/vestibular); turning on light for 30 secs (visual). Basal values of respiratory rate (90±5 and 88±8 cpm) and heart rate (377±10 and 354±10 bpm) did not differ between two groups. In Group 1 studied post-vehicle, respiratory rate raised transiently by 105±27, 150±27 and 183±15.03 cpm after tap, move and light stimuli respectively. Pharmacological inhibition of the DMH suppressed these responses by 92, 83 and 90% (tap/move/light). In Group 2 studied post-vehicle, respiratory rate raised transiently by 99±14, 118±23 and 165±20 cpm after tap, move and light stimuli respectively. Pharmacological inhibition of the medullary raphe suppressed these responses by 81, 75, and 71% (tap/move/light). Heart rate was not affected by alerting stimuli. Blockade of the DMH or raphe region had no effect on basal levels of respiratory or heart rate. We conclude that DMH and MR are not involved in the control of respiratory rate at rest, but their integrity is essential for mediating tachypnoeic arousal responses.

## POS-TUE-095

**IMMUNOHISTOCHEMICAL COMPARISON OF NEURONS IN THE GUINEA PIG AND HUMAN NODOSE GANGLION**McGovern A.E., Lockett J., Weir K.A. and Mazzone S.B.  
School of Biomedical Sciences, University of Queensland.

**PURPOSE:** In guinea pigs, defensive coughing is mediated by mechanosensitive neurons (cough receptors) that originate in the nodose ganglia. These neurons are characterised by the expression of neurofilament, alpha3 Na<sup>+</sup>/K<sup>+</sup> ATPase, and the transporters NKCC1, VGlut1 and VGlut2. Whether cough receptors are unique to guinea pigs is unknown. As an initial investigation into this question we compared the characteristics of neurons in the guinea pig and human nodose ganglia. **METHODS:** Male Hartley guinea pigs (n=5, 230-330g) were perfusion fixed with 4% PFA prior to harvesting nodose ganglia. Ganglia from humans were removed from embalmed donor cadaveric specimens (n=3, 2M:1F, 57-92 years). Cryostat cut slide mounted sections (12-16µm) were processed for neurofilament, alpha3 Na<sup>+</sup>/K<sup>+</sup> ATPase, NKCC1, VGlut1 and VGlut2 immunofluorescence or immunoperoxidase. **RESULTS:** Guinea pig nodose cells could broadly be categorised as having small (50-90µm, mean 87.3±4.5), medium (100-150µm, mean 122.2±5.8) or large (160-190µm, mean 173.4±4.6) perimeters. NKCC1 and VGlut2 labelled cells of all somal sizes whereas each of the other markers labelled subsets of cells with a highest relative frequency in the medium or large somal size range. The size of cells was similarly distributed in human nodose ganglia (60-170µm). Robust immunostaining for neurofilament, VGlut1 and VGlut2 was evident in many small, medium and large cells whereas only faint (but detectable) staining for alpha3 Na<sup>+</sup>/K<sup>+</sup> ATPase and NKCC1 was observed. **CONCLUSIONS:** These data indicate that cells in human nodose ganglia display some characteristics similar to guinea pigs. Markers that characterise guinea pig cough receptors are expressed by human nodose neurons, providing initial evidence that a comparable afferent neuron may exist.

## POS-TUE-094

**BRAIN ACTIVATION ASSOCIATED WITH EVOKED COUGH IS LESS THAN THE SUM OF ITS PARTS**Mazzone S.B.<sup>1</sup>, Cole L.J.<sup>2</sup>, Ando A.<sup>2</sup> and Farrell M.J.<sup>2,3</sup><sup>1</sup>School of Biomedical Sciences, University of Queensland. <sup>2</sup>Florey Neuroscience Institutes. <sup>3</sup>Centre for Neuroscience, University of Melbourne.

**PURPOSE:** Cough occurs in response to airways irritation and can be initiated voluntarily in the absence of airways stimulation. It has been proposed that supramedullary brain regions are likely to contribute to control of evoked cough through the facilitation or inhibition of brainstem reflex pathways. Using functional brain imaging, we put this putative model to the test. **METHODS:** Blood oxygen level-dependent (BOLD) contrast images were acquired using a 3T Siemens scanner from healthy participants (n=15) after inhalation of saline, inhalation of capsaicin without cough (cough suppression), inhalation of capsaicin with cough (evoked cough), and voluntary cough after inhalation of saline. BOLD signals were analysed to identify changes associated with cough, capsaicin stimulation and their interactions. **RESULTS:** Contrary to the hypothesised effect, the positive interaction of cough and capsaicin stimulation did not implicate brain activation uniquely associated with evoked cough. However, the negative interaction revealed brain activations that may be requisite for the initiation of voluntary cough and suppression of evoked cough (inferior frontal gyrus, SMA, cingulate cortex) as well as ongoing sensations associated with capsaicin in the airways (insula, orbitofrontal cortex) ( $p_{\text{corrected}} < 0.05$ ). **CONCLUSIONS:** The facilitation of evoked cough in response to airways irritation is not dependent on regional brain activation in addition to the activation that accompanies a voluntarily initiated cough. Indeed, voluntary cough and suppression of evoked cough are associated with brain activation in excess of evoked cough. This outcome suggests that evoked cough is a default motor pattern that does not require active facilitation by higher order centres, but is under higher order inhibitory influences.

## POS-TUE-096

**DIGIGAIT, A USEFUL METHOD ASSESSING SUBTLE GAIT ABNORMALITIES IN NEDD4 TRANSGENIC MICE**Bongiorno D.<sup>1,2</sup>, Petratos S.<sup>1,2</sup>, Kumar S.<sup>3</sup> and Poronnik K.<sup>1,2</sup><sup>1</sup>School of Medical Sciences. <sup>2</sup>Health Innovation Research Institute (HIRI). <sup>3</sup>Hanson Institute, Centre for Cancer Biology.

**Introduction:** Nedd4 (Neuronally Expressed Developmentally Down-regulated 4) is an E3 ubiquitin ligase that has an important role in the central nervous system (CNS). A recent study in embryonic Nedd4(-/-) mice found a decrease in size and number of motor neurons compared to controls<sup>1</sup>. However Nedd4 knockout (KO) mice are perinatal lethal, and thus assessment of motor function is only possible in Nedd4 heterozygous(+/-) mice. This may not be possible using traditional methods such as Rotarod, thus a novel method such as DigiGait may be more beneficial to investigate subtle motor changes. **Aim:** To identify if Nedd4 (+/-) display impaired motor function. **Methods:** Male Nedd4 (+/-) and wildtype (WT) mice were trained and then tested for the latency to fall of the rotarod. (2x2min accelerating and 1x5min from 4rpm-40rpm). For DigiGait analysis mice ran on a motorized transparent treadmill at two speeds 15cm/s and 20cm/s. **Results:** There was no significant difference in latency to fall (s) between WT (162±42, n=9) and Nedd4 (+/-) (166±46, n=8). DigiGait analysis showed that at 20cm/sec, hindpaw stance was significantly reduced in Nedd4 (+/-) compared to WT (0.15±0.005 and 0.17±0.006), as was the time spent in the propulsion phase (0.12±0.003 and 0.14±0.006). Nedd4 (+/-) paw angle was significantly reduced in both hindpaw and forepaw, at both speeds tested. **Conclusion:** This study has demonstrated the utility of DigiGait as an excellent tool in identifying subtle phenotypic changes in transgenic mice. 1.Liu Y, Oppenheim RW, Sugiura Y and Lin W. (2009). Developmental Biology, 330(1), pp153-166.



## POS-TUE-097

**RECEPTORS IN MOTION: RASTER IMAGE CORRELATION SPECTROSCOPY (RICS) ANALYSIS OF PEPTIDE RECEPTOR AND MEMBRANE MOBILITY**

DeGraaf Y.C.<sup>1</sup>, Clarke J.N.<sup>1</sup>, Gratton E.<sup>2</sup>, Digman M.<sup>2</sup>, Thomas W.G.<sup>3</sup> and Gibbins I.L.<sup>1</sup>

<sup>1</sup>Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, Australia. <sup>2</sup>Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, USA. <sup>3</sup>School of Biomedical Sciences, University of Queensland, Australia.

Neuronal cell membranes are dynamic structures in which peptide receptors form distinct domains. Peripheral sympathetic neurons express different G-protein coupled receptors (GPCRs) regulating a common set of ion channels that probably co-exist in the same membrane domains. However, it is not known how domains enriched in functionally-linked receptors and channels are maintained in a dynamic membrane structure. We have begun to investigate this problem using analytical confocal imaging techniques with very high spatial and temporal resolution to examine cell lines expressing GPCRs. High resolution confocal images have been analysed with Raster Image Correlation Spectroscopy (RICS) and its derivatives. We have used CHO cells transfected with angiotensin II receptors (type 1A; AT-1AR) modified with enhanced green fluorescent protein (eGFP) on their intracellular C-terminus, combined with angiotensin II coupled with Alexa647 fluorophore. Images were collected in photon counting mode on a Leica SP5 confocal microscope or an Olympus FluoView 1000 and analysed with SimFCS 2.0. High resolution imaging revealed a surprising amount of mobility in the cell membrane in addition to processes associated with the internalisation of agonist bound to receptors. Membrane regions rich in AT-1AR-eGFP displayed rapidly moving filopodia with an effective diffusion coefficient for the receptors of 0.1-3  $\mu\text{m}^2/\text{s}$  (n=6), equivalent to a linear velocity of about 1  $\mu\text{m}/\text{s}$ . Our results to date suggest that the analysis of agonist-receptor interactions, at least in cell lines, will be significantly confounded by the consequences of membrane mobility.

## POS-TUE-098

**CAN VOLUME TRANSMISSION REALLY WORK? MODELLING EXTRACELLULAR DIFFUSION OF SUBSTANCE P**

Gibbins I.L., Goh S.X., DeGraaf Y.C. and Clarke J.N.

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

The concept that neuropeptides can act non-synaptically via volume transmission has been debated for more than 20 years. Fluorescence correlation spectroscopy (FCS) combined with scanning laser confocal microscopy permits direct measurement of diffusion properties of neuropeptides labelled with a fluorescent tag. We have used FCS to examine diffusion of substance P (SP) labelled with Oregon Green-488 (SP-OG) in the neighbourhood of CHO cells expressing neurokinin-1 (NK1) receptors. We used a Leica SP5 confocal microscope fitted with dual avalanche photodiode detectors coupled to an ISS photon counting and FCS system. Our FCS data provide a diffusion coefficient for SP-OG of around 200  $\mu\text{m}^2/\text{s}$  in balanced salt solution. We used the FCS data to model how SP might diffuse through the extra-cellular environment between neurons. Neurotransmitter diffusion was modelled in MatLab (R12) to solve differential equations with variables including the apparent diffusion coefficient of the peptide, the tortuosity of the diffusion path, the volume fraction available for peptide diffusion, the number of potential release sites and the amount of SP released. The models suggest that SP concentrations follow a complex spatio-temporal profile following neural release. After a single release event, SP concentration falls off rapidly in space and time making long range transmission would be unlikely. However, after multiple releases, even at frequencies below 10Hz, extracellular SP concentrations remain in the nM- $\mu\text{M}$  range up to 20  $\mu\text{m}$  from the release sites for 20 seconds or more. This concentration range can activate neuronal receptors NK1 receptors, supporting the feasibility of volume transmission. The reliability of such transmission depends critically on the number and disposition of release sites.

## POS-TUE-099

**PREPULSE INHIBITION OF THE STARTLE RESPONSE PARADIGM IN NEDD4 MICE**

Guille V.<sup>1,2,3</sup>, Bongiorno D.<sup>1,2</sup>, Petratos S.<sup>1,2</sup>, Kumar S.<sup>4</sup> and Poronnik P.<sup>1,2</sup>

<sup>1</sup>School of Medical Sciences. <sup>2</sup>Health Innovation Research Institute (HIRI). <sup>3</sup>Neuro Research Services (NRS). <sup>4</sup>Hanson Institute, Centre for Cancer Biology.

**Introduction:** Nedd4 (neural precursor cell-expressed developmentally downregulated gene 4) is a cytoplasmic E3 ubiquitin ligase responsible for the targeted degradation of proteins through the ubiquitin-proteasomal pathway. Alterations in the function of the ubiquitin-proteasomal system have recently been implicated in the pathology observed in many neurodegenerative disorders such as Alzheimer Disease (AD) and Parkinson Disease (PD). Prepulse Inhibition (PPI) is a measure of sensory gating. Deficits of PPI reflect an inability to filter out unnecessary sensory information. Previous animal studies have indicated that the hippocampus and entorhinal cortex structures affected in mild AD, are involved in the regulation of PPI. It has also been reported that PD patients exhibited a deficiency in sensorimotor gating mechanisms. We therefore examined whether the Nedd4 heterozygote knockout mice (Nedd4<sup>+/-</sup>) exhibited a sensorimotor gating deficit in the PPI of the acoustic startle response paradigm. **Method:** Male transgenic Nedd4<sup>+/-</sup> (n = 12) and wild type (WT; n = 8) 8-12 week-old mice were used. Prepulse startle responses were assessed using the SR-Lab Startle Response System. **Results:** No significant differences were found in PPI between Nedd4<sup>+/-</sup> and WT mice. **Conclusion:** These data suggests that Nedd4<sup>+/-</sup> mice do not display abnormalities of sensorimotor gating and have similar ability to filter out unnecessary sensory information when compared to WT mice.

## POS-TUE-100

**p53 AND NDFIP1 REGULATE STRESS INDUCED EXOSOME RELEASE**

Putz U. and Tan S.-S.

Howard Florey Institute, University of Melbourne, Victoria 3010, Australia.

The ability to remove unwanted proteins involves ubiquitylation followed by degradation of the marked protein in the proteasome. An alternative mechanism for protein removal and trafficking is provided by exosomes, which are small vesicles (50–90-nm diameter) originating from late endosomes and multivesicular bodies (MVBs). Cells undergoing stress conditions show increased exosome release. p53 KO cells (n = 6) show a decrease in stress induced exosome release and a role for p53 target genes in exosome release was postulated. The activation of target genes of p53 is regulated by at least two members of the Nedd4 family of E3 ubiquitin ligases (NEDL1 and WWP1). Ndfip1 (Nedd4 family-interacting protein 1), an adapter protein for the Nedd4 family, is also involved in exosomal release and further plays a role in the export of specific proteins in exosomes. Overexpression of Ndfip1 in different cell lines (n = 10) enhances exosome release and stressed cells show an increase in exosomal release of Ndfip1. Ndfip1 KO cells show a similar phenotype to p53 KO cells regarding stress-induced exosome release: After the induction of stress, Ndfip1 KO cells are also unable to increase exosome release, like p53 KO cells (n = 10). Therefore p53 and Ndfip1 may share a common pathway leading to increased exosome release after stress. Given the positive roles of Ndfip1/Nedd4 in improving neuronal survival during brain injury, it is possible that increased exosome secretion provides a novel route for rapid sequestration and removal of proteins during stress.

## POS-TUE-101

**EXPLORING SOLUBLE ANTAGONISTS OF EPHA4 AS A POTENTIAL THERAPEUTIC FOR SPINAL CORD INJURY**

**Spanevello M.D.<sup>1,2</sup>**, Tajouri S.I.<sup>2</sup>, Turnley A.M.<sup>3</sup>, Boyd A.W.<sup>1,4</sup> and Bartlett P.F.<sup>2</sup>

<sup>1</sup>Queensland Institute of Medical Research, HERSTON, QLD, 4029.

<sup>2</sup>Queensland Brain Institute, University of Queensland, ST LUCIA, QLD, 4072. <sup>3</sup>Centre for Neuroscience, University of Melbourne, PARKVILLE, VIC, 3010. <sup>4</sup>School of Medicine, University of Queensland, ST LUCIA, QLD 4072.

The Ephs and the ephrins comprise a receptor:ligand system capable of bidirectional signalling with important roles in cell migration and segregation during development. EphA4, a unique Eph receptor capable of interacting with both classes of ephrins, is an important regulator of CNS development and function. Mice lacking EphA4 receptors show a decrease in the gliotic response and remarkable functional recovery following a lateral hemisection injury of the spinal cord. Exploiting the ability of Fc fusion proteins to block Eph:ephrin signalling, we have now generated soluble antagonists of EphA4 by fusing the extracellular domain of EphA4 or its high-affinity ligand, ephrin A5, to the human IgG1 Fc domain. Previous experiments of hemisectional injury in wild type mice with a two-week treatment permits significant functional improvement and substantial axon regeneration in 6 weeks. Here we present data from two additional cohorts. Extending the duration of treatment from two weeks (n=11) to four weeks (n=10) does not substantially improve functional outcome. Furthermore, mice recovering for periods of 8 weeks (n=41), 12 weeks (n=27) and 6 months (n=13) display maximal recovery within 8-12 weeks of injury and no functional deterioration at 6 months. These results demonstrate that a two-week treatment and an 8-week recovery period are optimal for assessing the therapeutic benefit of EphA4-Fc in spinal cord injuries. EphA4-Fc is continually demonstrating beneficial outcomes for treating spinal cord injuries and proving to be an important therapeutic opportunity for many CNS injuries and diseases.

## POS-TUE-103

**ESTABLISHING AN *IN VITRO* MODEL TO INVESTIGATE MICROGLIA INFLUENCE ON HIPPOCAMPAL NEURAL PRECURSORS**

**Vukovic J.**, Walker T.L. and Bartlett P.F.

The Queensland Brain Institute, The University of Queensland, Brisbane.

Microglia are capable of secreting factors that can either stimulate or inhibit proliferation and differentiation of neural precursor cells. The activation status and secretory profile of microglia thus partly shape the molecular microenvironment of the neurogenic niche, which in turn can influence neurogenesis under both normal and pathological conditions. To date, however, detailed understanding of the influence of microglia on neural precursors has been hampered by the absence of an assay to study microglia-neural precursor interactions under defined conditions. The main aim of this study was therefore to establish a quantitative *in vitro* model of hippocampal microglia and neural precursor cell interactions. In this study, we took advantage of CX3CR1<sup>+/GFP</sup> mice which express green fluorescent protein (GFP) in all cells of monocytic lineage, including brain microglia. Neurosphere frequency of wild-type and CX3CR1<sup>GFP/GFP</sup> (i.e. CX3CR1-deficient) mice were ascertained to determine whether targeted deletion of one CX3CR1 allele interfered with neural precursor proliferation. No difference in neurosphere-forming frequency was observed between wild-type and CX3CR1-deficient mice (n=3) even in assays which activated latent precursor with high KCl (Walker et al. 2008). Interestingly, however, KCl depolarisation increased total microglia numbers in neurosphere cultures by 27%. GFP<sup>+</sup> microglia were sorted from CX3CR1<sup>+/GFP</sup> hippocampi using FACS with an average 2218 ± 820 live microglia isolated per hippocampus (n=3). Isolated cells displayed the well-defined microglia phenotype as determined by expression of the markers CD11b<sup>+</sup> and CD45<sup>dim</sup>. Ongoing co-culturing experiments indicate a dose-dependent effect of microglia on the neurosphere-forming frequency from neural progenitors. Walker TL, White A, Black DM, Wallace RH, Sah P, Bartlett PF (2008) J Neurosci 28:5240.

## POS-TUE-102

**THE ROLE OF SOCS2 IN NEURITE MORPHOLOGY OF CULTURED DORSAL ROOT GANGLIA NEURONS**

**Uren R.T.** and Turnley A.M.

Centre for Neuroscience, University of Melbourne, Melbourne, Victoria, 3010, Australia.

Overexpression of Suppressor of Cytokine Signalling-2 (SOCS2) promotes increases in neurite length and neurite number in PC12 cells and cortical neurons. The mechanisms by which SOCS2 regulates the signals that control neurite outgrowth and neuronal differentiation are unresolved but appear to involve Trk receptors. To examine potential effects of SOCS2 and Trk interactions in primary neurons, morphology of TrkA expressing Dorsal Root Ganglion (DRG) neurons from SOCS2 overexpressing SOCS2 transgenic (SOCS2-Tg) mice were compared to wildtype neurons. DRGs were obtained from 1 day old post-natal wildtype (WT) (n=3) or SOCS2-Tg mice (n=4) pups and dissociated neurons were cultured for 4 hours with 50 ng/mL NGF. DRG neurons were fixed and immunostained for the neuronal marker beta III tubulin. Neurons were classified based on neurite morphology and the relative proportions of DRG neurons in each subpopulation were compared between WT and SOCS2-Tg genotypes. DRG neurons from SOCS2-Tg mice demonstrated an increased proportion of neurons with complex neurite morphology. Conversely, a decrease in the proportion of neurons with immature or absent neurites in the SOCS2-Tg cultures was observed. No change was observed in the proportion of neurons bearing simple neurite morphology between genotypes. Overexpression of SOCS2 promotes the culture of a neuronal subpopulation from Dorsal Root Ganglia with increased neurite complexity and a reduction in the early prevalence of neurons without neurites and those bearing immature neurites. This finding may reflect enhanced neurite outgrowth or branching in response to treatment with nerve growth factor *in vitro* or changes in the relative abundance of DRG neuronal subtypes in the developing SOCS2 transgenic mouse due to survival or differentiation effects.

## POS-TUE-104

**METALLOTHIONEINS INDUCE GROWTH CONE CHEMOTAXIS**

**Landowski L.M.**, Chung R.S., Gasperini R., Small D.H., West A.K. and Foa L.

Menzies Research Institute, University of Tasmania, Hobart, 7001, Tasmania, Australia.

Disruption of neuronal networks can occur in brain injury or as a consequence of neurodegenerative disease. The metallothionein (MT) family of proteins are candidates for enhancing neuronal repair, and are known to be crucial in functional recovery after CNS injury. MTIII has been shown to inhibit neuronal outgrowth. MTI and MTII (MTI/II) exhibit neuroprotective, anti-apoptotic and growth-promoting effects. The neuronal response to MTI/II is characterised by significant increase in neuronal outgrowth and sprouting of neurites. In this study, using the well established growth cone turning assay, the acute response of actively navigating embryonic (E15-18) rat sensory neurons to a gradient of MTI/II and MTIII was measured *in vitro*. Neurites responded to MTI/II by chemoattraction, and MTIII by chemorepulsion ( $8.7^\circ \pm 1.3$ , n=12 and  $-13.8^\circ \pm 1.9$ , n=14 respectively, compared to PBS control,  $-1.1^\circ \pm 1.8$ ; P<0.02). It was found that LRP-receptor inhibitor, RAP, abrogated the chemotactic effect of MTs, suggesting that MT chemotaxis occurs via binding LRP receptors, such as megalin. Immunocytochemical staining of growth cones established that megalin is distributed appropriately in growth cones for chemical sensing. MT chemotaxis is dependent on the presence of extracellular calcium: in low calcium media, a molecular switch turns chemoattraction into chemorepulsion, and vice versa, whereas bathing neurons in calcium free media abolishes MT-mediated chemotaxis altogether. Understanding the mechanisms by which MTs elicit this response in neuronal growth cones has important implications in future therapeutic developments. The ability of MTs to induce chemotaxis, coupled with their neuroprotective and neuroregenerative properties, may render them an effective modality in rescuing damaged neurons and assisting re-innervation and repair at the site of injury.

## POS-TUE-105

**FUNCTIONAL DOPAMINERGIC DIFFERENTIATION OF PURIFIED MICE MÜLLER CELLS IN CULTURE**

Stutz B.<sup>1,2</sup>, Conceicao F.S.L.<sup>3</sup>, Cadilhe D.V.<sup>3</sup>, Gardino P.F.<sup>1</sup>, Rehen S.K.<sup>3</sup>, Houzel J.C.<sup>3</sup> and de Mello F.G.<sup>1</sup>

<sup>1</sup>Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro. <sup>2</sup>School of Biomedical Sciences and Pharmacy, University of Newcastle. <sup>3</sup>Institute of Biomedical Sciences, Federal University of Rio de Janeiro.

Müller cells constitute the main glial cell type in the retina and span the tissue from the inner to the outer limiting membranes. Several functions have been attributed to these cells including structural and nutritional roles as well removal of ions and neurotransmitters from the extracellular space. The potential of Müller cells to actively participate in cellular communication within the nervous system has been recently uncovered. Moreover, it has been suggested that this type of cell can generate neurons under appropriate conditions. Knowledge of the mechanisms controlling this particular differentiation allow production of cells that could be used in potential therapy against some neurodegenerative disorders. In the present work, we evaluated the capability of Müller cells to be used as a source of dopaminergic cells and the factors that influence this process. We have shown that Müller cells in culture (n=3) not only express dopaminergic markers proteins such as the dopamine transporter (DAT), tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and the transcription factor for dopaminergic differentiation Nurr1, but they are also able to produce dopamine *in vitro* (n=5), reaching values of 50 ± 10nmoles or 65 ± 8nmoles, when treated either with forskolin 10µM or PACAP38 10nM, respectively. A mouse model of Parkinson's Disease is obtained with stereotactic injection of 6-OH-DA in the striatum. In these mice, injection of functional dopaminergic differentiated Müller cells clearly reduced the typical rotational behavior presented by the animals (from 15±2 rpm to 2±1 rpm) (n=7).

## POS-TUE-106

**LOW AND HIGH AFFINITY CATECHOLAMINE BINDING SITES IN TYROSINE HYDROXYLASE SHOW STRUCTURAL SIMILARITY**

Briggs G.D., Gordon S.L., Dunkley P.R. and Dickson P.W.  
School of Biomedical Sciences and Pharmacy, and Hunter Medical Research Institute, Faculty of Health, University of Newcastle, Callaghan, NSW 2308, Australia.

**PURPOSE:** Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of the catecholamines dopamine, noradrenaline and adrenaline and controls the rate of production of catecholamines in cells. Short-term control of TH activity is achieved through a combination of feedback inhibition by the catecholamines and reactivation by phosphorylation. Catecholamines bind TH irreversibly and with high affinity to inhibit the enzyme. Phosphorylation of TH allows dissociation of bound catecholamine, thereby increasing enzyme activity. We have identified a second catecholamine inhibitory site which is readily reversible and functions independently of the phosphorylation state of the enzyme, thus controlling the level of cytosolic catecholamines under both basal and stimulated conditions. In this study TH mutants were generated to determine the position of the novel low affinity catecholamine binding site in TH. **METHODS:** The crystal structure of the TH active site was used to identify residues responsible for low affinity site. The dopamine dependent inhibition of TH activity in wild-type and active site TH mutants was measured. **RESULTS:** The IC<sub>50</sub>s for dopamine inhibition through the low affinity site in TH mutants Tyr371Phe and Glu332Asp were 70-fold and 10-fold higher than wild-type respectively (p<0.005, n=3). Catecholamine bound to the high affinity site produced a 10-fold increase in the Km for the cofactor in wild-type TH. In Tyr371Phe, Glu332Asp and Ala297Leu this inhibitory effect was absent. **CONCLUSIONS:** The results from this work indicate that the low affinity catecholamine binding site is localised to the active site of TH and is likely to be structurally similar to the high affinity site.

## POS-TUE-107

**MECHANISMS OF PHOSPHORYLATION-SENSITIVE CAMKII TARGETING**

Skelding K.A.<sup>1</sup>, Xue J.<sup>2</sup>, Suzuki T.<sup>3</sup>, Verrills N.M.<sup>1</sup>, Dickson P.W.<sup>1</sup> and Rostas J.A.P.<sup>1</sup>

<sup>1</sup>School of Biomedical Sciences and Pharmacy and Hunter Medical Research Institute, University of Newcastle, Callaghan, NSW 2308, Australia. <sup>2</sup>Cell Signalling Unit, Children's Medical Research Institute, Wentworthville, NSW 2145, Australia. <sup>3</sup>Department of Neuroplasticity, Shinshu University Graduate School of Medicine, Matsumoto, Japan.

Calcium/calmodulin stimulated protein kinase II (CaMKII) is an important regulator of neuronal function. The biological properties of CaMKII are regulated by multi-site autophosphorylation and targeting to cellular microdomains through interactions with specific proteins. The role of autophosphorylation at Thr286 has been well characterised and shown to regulate CaMKII function by altering CaMKII activity and CaMKII targeting. We have identified a new autophosphorylation site at Thr253, which regulates CaMKII function exclusively through targeting. To identify which regions of CaMKII are responsible for binding to interacting proteins, short peptides corresponding to different regions of CaMKII (α-CaMKII 310-320, 249-258, 282-291) were examined for their ability to inhibit CaMKII binding to a panel of known binding partners in a semi-quantitative overlay binding assay (n=3-6). We observed highly selective inhibition profiles indicating that: 1. One region of CaMKII can interact with more than one binding partner; 2. Autophosphorylation induced changes in CaMKII binding can change the region of CaMKII involved in the interaction and, by implication, also the region of the binding protein involved in this interaction. We have also shown that non-phosphorylated CaMKII and CaMKII phosphorylated at Thr253, but not Thr286, binds neurogranin. However, phosphorylation of neurogranin by PKC completely abrogates binding (neurogranin is not a CaMKII substrate). These results imply that phosphorylation-induced alterations in the targeting of CaMKII can mediate crosstalk between different signalling pathways leading to changes in the signalling pathways into which CaMKII is linked, thereby altering functional outcomes.

## POS-TUE-108

**REGULATION OF PROLIFERATION OF NEUROBLASTOMA CELLS BY CAMKII**

Skelding K.A., Verrills N.M., Dickson P.W. and Rostas J.A.P.  
School of Biomedical Sciences and Pharmacy and Hunter Medical Research Institute, University of Newcastle, Callaghan, Australia.

CaMKII is an important regulator of a variety of cellular functions including cell growth and proliferation. The biological properties of CaMKII are regulated by phosphorylation and targeting to cellular domains via interactions with proteins. The roles of phosphorylation at T286 have been well characterised. We have identified a new phosphorylation site at T253 whose phosphorylation *in vivo* is dynamically regulated independently of T286 phosphorylation. We have demonstrated that, following transfection of the SHSY5Y neuroblastoma cell line with wild type CaMKII (WT), T286D-CaMKII (mimicking phosphorylation at T286), or T253D-CaMKII (mimicking phosphorylation at T253), the morphology and growth rate of these cells is differentially altered (n=3). Transfection with WT approximately doubled the growth rate without any alteration in cell morphology. The effect of transfection with T286D-CaMKII was identical to that produced by WT showing that phosphorylation at T286 had no effect on growth rate or cell morphology. By contrast, transfection with T253D-CaMKII dramatically reduced cell growth and altered cell morphology. To identify the mechanism behind this T253D-CaMKII mediated block in proliferation, we examined changes in endogenous CaMKII at various stages of the cell cycle (n=3). We found that while there is no change in total CaMKII expression, there appears to be a marked decrease in T253 phosphorylation at the G2/M border. These results strongly suggest that phosphorylation of CaMKII at T253 is involved in regulating neuronal cell growth and morphology independently of phosphorylation at T286, and clearly identifies functional consequences following T253 phosphorylation.



## POS-TUE-109

**CHEMOKINES AND INFLAMMATORY MEDIATORS REGULATE NEURAL PROGENITOR CELL DIFFERENTIATION**

**Turbić A.**, Leong S.Y. and Turnley A.M.  
Centre for Neuroscience, Level 7, West Wing, Medical Building, The University of Melbourne, Parkville VIC 3050.

Adult neural progenitor cells (NPCs) respond to injury or disease of the CNS by migrating to the site of neural damage and/or differentiating locally to replace lost neurons, astrocytes and oligodendrocytes. Factors that mediate this injury induced NPC response include pro-inflammatory cytokines, such as tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) and interferon- $\gamma$  (IFN $\gamma$ ), which we have shown previously promotes neuronal differentiation, as well as chemokines. RT-PCR was used to compare expression of CXC, CC, C and CX3C family chemokines and their receptors in normal adult mouse brain, and in cultured NPCs in response to IFN $\gamma$  and TNF $\alpha$ . Basal expression of several chemokines and their receptors was found, predominantly in neurogenic regions, with OB>SVZ>hippocampus and little or no expression in non-neurogenic regions, such as cortex. Treatment of SVZ-derived NPCs with IFN $\gamma$  and TNF $\alpha$  (alone and in combination) resulted in significant up- or down-regulation of expression of specific chemokines, with CXCL1, CXCL9 and CCL2 most highly upregulated and CCL19 downregulated. Unlike IFN $\gamma$ , chemokine treatment of NPCs in vitro had little or no effect on migration, survival or proliferation. Neuronal differentiation was promoted by CXCL9 or CCL21, while glial differentiation was modestly promoted by CCL19 and CCL21 and blocked by IFN $\gamma$ . IFN $\gamma$  (+/- chemokines) promoted oligodendrocyte maturation but had little effect on overall oligodendrocyte differentiation. Therefore, not only do NPCs express chemokine receptors, they also produce several chemokines, particularly in response to inflammatory mediators. This suggests that autocrine or paracrine production of specific chemokines by NPCs in response to inflammatory mediators may regulate differentiation into mature neural cell types and may alter responsiveness to CNS injury or disease. Keywords: neural progenitor cells, inflammatory mediators, chemokines

## POS-TUE-111

**CALCITONIN GENE-RELATED PEPTIDE (CGRP) ANTAGONISTS DECREASE THE EXCITATORY INPUT OF PRIMARY AFFERENT INPUT ONTO TRIGEMINAL SUBNUCLEUS CAUDALIS NEURONS**

**Williams M.W.** and Jennings E.A.  
University of Melbourne, Department of Anatomy & Cell Biology.

Calcitonin gene-related peptide (CGRP) is the primary neuropeptide released during migraine via activation of trigeminovascular neurons. Whilst much is known about the peripheral vasoactive role of CGRP, less is known about the effects CGRP may have on central nociceptive neurons in the trigeminal nucleus caudalis. Using whole-cell patch-clamp electrophysiological recordings in brainstem slices, we examined the effect of the nonpeptide CGRP receptor antagonist BIBN4096BS on evoked excitatory postsynaptic currents (eEPSC) in second order trigeminal neurons. In response to electrically stimulated trigeminal primary afferent fibres, bath application of BIBN4096BS decreased mean ( $\pm$  SEM) eEPSC amplitude by  $22.6 \pm 5.3\%$  ( $n=12$ ;  $p=0.012$ ). The results of the current study suggest that a proportion of excitatory neurotransmission is mediated by CGRP receptors at the first synapse in the spinal trigeminal nucleus. These results suggest that at least part of the mechanism of BIBN4096BS in clinical trials occurs at the primary afferent-second order central synapse and strengthens the idea that central inhibition CGRP receptors may be effective in the treatment of migraine pain.

## POS-TUE-110

**GALANIN POTENTIATES AMYLASE SECRETION BY MOUSE PANCREATIC LOBULES BUT NOT BY ISOLATED ACINAR CELLS**

Bazargan M.<sup>1</sup>, Barreto S.G.<sup>1</sup>, Schlothe A.C.<sup>1</sup>, Carati C.J.<sup>2</sup>, Toouli J.<sup>1</sup> and **Saccone G.T.P.<sup>1</sup>**

<sup>1</sup>Department of General and Digestive Surgery, Flinders University.  
<sup>2</sup>Department of Anatomy and Histology, Flinders University.

Galanin is implicated in the pathogenesis of acute pancreatitis. Many rodent models of acute pancreatitis use supramaximal concentrations of caerulein to induce the disease. The effect of galanin on amylase secretion under these conditions is unclear. We hypothesised that galanin modulates pancreatic amylase secretion evoked by a supramaximal concentration of caerulein ( $10^{-7}$ M) by a direct action on the acinar cells (AC). We compared the effect of exogenous galanin on amylase secretion from pancreatic lobule and AC preparations evoked by  $10^{-7}$ M caerulein. Lobules and AC were prepared from mouse pancreata by standard collagenase digestion techniques. Lobules or AC ( $n=5-7$  preparations) were incubated with galanin ( $10^{-13}$ – $10^{-7}$ M), caerulein ( $10^{-12}$ – $10^{-7}$ M), alone or in combinations for 60 min at 37°C. Control lobules or AC were incubated in medium alone. Amylase activity into the incubation medium was measured and expressed as % of total amylase (medium plus lobules/AC). Caerulein stimulated amylase secretion from lobules and AC in a dose-dependent manner ( $P<0.05$ ). The peak secretion from lobules and AC was 170% and 330%, respectively, of control and evoked by  $10^{-10}$ M caerulein in both preparations. Secretion then declined with increasing concentration in both preparations. Galanin alone did not influence basal amylase secretion from lobules and AC. Caerulein ( $10^{-7}$ M) alone stimulated amylase secretion from lobules to 124% of control, whereas co-incubation with galanin ( $10^{-12}$ M– $10^{-7}$ M) potentiated caerulein-stimulated amylase secretion up to 160% of control ( $P<0.05$ ). In contrast, galanin had no effect on the caerulein-stimulated amylase secretion from AC. We conclude that galanin does not act directly on AC to regulate pancreatic amylase secretion.

## POS-TUE-112

**GALANIN AND ITS RECEPTORS ARE EXPRESSED IN THE WHOLE MOUSE PANCREAS, ISOLATED ACINAR AND ISLET CELLS**

**Bazargan M.<sup>1</sup>**, Hussey D.J.<sup>1</sup>, Leong M.<sup>1</sup>, Peiris H.<sup>2</sup>, Keating D.J.<sup>2</sup>, Carati C.J.<sup>3</sup>, Toouli J.<sup>1</sup> and **Saccone G.T.P.<sup>1</sup>**

<sup>1</sup>Department of Surgery, Flinders University. <sup>2</sup>Department of Human Physiology, Flinders University. <sup>3</sup>Department of Anatomy and Histology, Flinders University.

Galanin is a neurotransmitter/neuromodulator associated with the pancreatic vasculature in many species. Galanin also modulates pancreatic exocrine secretion. Galanin acts via 3 known receptors, galanin receptor 1 (GalR1), GalR2 and GalR3. The galanin receptor expression in the pancreas however is not fully described. We aimed to establish if galanin and its 3 receptors are expressed in normal mouse pancreas, acinar and islet cells. Pancreata were rapidly harvested from mice. Acinar and islets cells were isolated from mouse pancreas by standard protocol specifically techniques. Extraction of total RNA used a Trizol designed for pancreatic tissue. The expression of galanin, GalR1, GalR2 and GalR3 mRNA was determined using real-time reverse transcription-polymerase chain reaction (RT-PCR) with primers designed specifically for these transcripts. 18S rRNA was used as a housekeeping gene for normalisation of expression data. In the whole pancreas ( $n=3$ ), expression of galanin and its 3 receptors was detected. GalR3 showed the highest expression followed by GalR1 then GalR2. In islet cells ( $n=2$  preparations) GalR3 was highly expressed whereas GalR1, GalR2 and galanin appear to be poorly expressed. By comparison with islet expression, the acinar cell ( $n=3$  preparations) expression of the 3 galanin receptors was very low, but surprisingly, galanin was well expressed. We conclude that the 3 galanin receptors are present in the mouse pancreas, but their relative expression varies with the different cell types studied. The poor expression of galanin receptors on acinar cells is consistent with our finding that galanin does not modulate amylase secretion by directly acting on acinar cells.

## POS-TUE-113

# ANATOMY AND PHYSIOLOGY OF GABA-ERGIC FEEDBACK EXCITATION IN PARVALBUMIN EXPRESSING INTERNEURONS OF THE BASOLATERAL AMYGDALA

**Spampanato J.**, Sullivan R.K.P., Bartlett P. and Sah P.  
Queensland Brain Institute, The University of Queensland, St. Lucia, QLD, 4072.

Parvalbumin expressing interneurons of the basolateral amygdala have previously been shown to form an excitatory feedback excitation loop involving at least one glutamatergic cell. This circuit can be identified in juvenile mice at post-natal day 21 and is preserved in mature adults. GABAergic interneurons with identified feedback excitation were filled with biocytin and developed for light and electron microscopy. Axonal length and arborization varied greatly from cell to cell, however in each case (n=32) the presynaptic interneuron was observed to make traditional axo-somatic basket synapses as well as axo-dendritic and axo-axonic synapses confirmed by co-labeling with the axon initial segment marker Ankyrin-G. Axo-axonic synapses also varied between the more classical "cartridge" type and single synaptic contacts. We tested the hypothesis that the Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> cotransporter (NKCC1), which maintains a high internal [Cl<sup>-</sup>] by pumping Cl<sup>-</sup> into the cell along the Na<sup>+</sup> gradient, is required for the excitatory GABAergic feedback. Application of the antagonist bumetanide had no effect, suggesting that the feedback excitation is due to the lack of K<sup>+</sup>/Cl<sup>-</sup> cotransporter (KCC2), which sets a low internal [Cl<sup>-</sup>] by extruding Cl<sup>-</sup> along the K<sup>+</sup> gradient. This was confirmed by immunohistochemical labeling of KCC2, which appeared to be present in dendrites but not in initial segments.

## POS-TUE-114

# PROPERTIES OF THE INTERCALATED NEURONS IN THE RODENT AMYGDALA

**Strobel C.E.L.** and Sah P.  
The University of Queensland, Queensland Brain Institute, 4072, Qld, Australia.

The intercalated cell masses (ITCs) of the amygdala are a cluster of interneurons located between the basolateral complex and central nucleus, the main input and output stations of the amygdala. These neurons play an important role during extinction of conditioned fear and act as feed forward interneurons for cells in the central amygdala. However, their physiological properties are little understood. Methods: Using immunohistochemistry and whole-cell recordings in acute slices we have characterised the ITCs in GAD67-EGFP transgenic mice. Results: Immunohistochemical staining for calretinin, calbindin, parvalbumin, somatostatin and cholecystokinin showed that there was no expression for calretinin, calbindin, parvalbumin and somatostatin. Some cells expressed cholecystokinin in cell bodies and nerve terminals. Two types of neurons (n=50) were found on electrophysiological recordings: 34 % (17/50) of ITCs showed clear adaptation during the course of the 700 ms current injection and 66 % (33/50) of neurons fired repetitively. Synaptic stimulation evoked large polysynaptic IPSCs in most neurons. However, paired recordings revealed a connection rate of only 13.4 % (4/30 pairs). This result shows that ITCs have a different phenotype from most interneurons, lacking the typical calcium binding proteins. In further studies we will investigate whether synapses onto the ITCs are plastic and if these cells could not only be a site for the expression of extinction but also a site for the storage of extinction memory.

## POS-TUE-115

# ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE MEDIAL AMYGDALA IN MICE

**Keshavarzi S.** and Sah P.  
Queensland Brain Institute, The University of Queensland.

**BACKGROUND:** The medial nucleus of the amygdala (MeA) is a subcortical structure that processes olfactory signals and plays a key role in regulating social, defensive and reproductive behaviours. However, the cellular and synaptic properties of MeA neurons have not been well characterized. **METHODS:** We have characterized neurons in the MeA of adult male GAD67-eGFP knock-in mice using immunohistochemistry and whole cell recordings in acute brain slices. Synaptic responses in these neurons were examined by stimulation of putative afferent olfactory inputs within the MeA external layer. **RESULTS:** Immunohistochemistry showed the presence of calbindin and calretinin in the MeA. However, there was no immunoreactivity for parvalbumin and somatostatin. The majority of calbindin positive cells were GFP<sup>+</sup>, whereas calretinin positive cells appeared to be largely GFP<sup>-</sup>. Both GFP<sup>+</sup> (n=36) and GFP<sup>-</sup> (n=57) cells could be divided into two types based on their responses to depolarising current injections: Repetitive firers (42% GFP<sup>+</sup>, 30% GFP<sup>-</sup>) and accommodating neurons (58% GFP<sup>+</sup>, 70% GFP<sup>-</sup>). There was no significant difference in the amplitude of afterhyperpolarizations between the two firing subtypes in either the GFP<sup>+</sup> or GFP<sup>-</sup> groups. Most cells possessed I<sub>h</sub> (80% GFP<sup>+</sup>, 50% GFP<sup>-</sup>) and I<sub>t</sub> (75% GFP<sup>+</sup>, 54% GFP<sup>-</sup>) which frequently gave rise to rebound spikes. Synaptic stimulation largely evoked monosynaptic glutamatergic responses that were mediated by both AMPA and NMDA receptors. A polysynaptic GABAergic input was often present in both GFP<sup>+</sup> and GFP<sup>-</sup> neurons. These results represent the first step towards understanding the internal circuitry of the MeA and its role in olfactory processing and social recognition.

## POS-TUE-116

# EFFECTS OF SENSORY STIMULI ON AMYGDALA NEURON ACTIVITY IN VIVO

**Windels F.**, Crane J. and Sah P.  
The Queensland Brain Institute.

Pavlovian fear conditioning involves the pairing of a non-aversive stimulus (conditioned stimulus, CS) with an aversive stimulus (unconditioned stimulus, US). The basolateral amygdala (BLA) is critical for fear conditioning, and convergence of CS and US-related inputs onto single BLA neurons is thought to lead to an enhanced synaptic response to CS presentations. Central to this hypothesis is that CS- and US-related inputs converge onto single BLA neurons. The strongest evidence for the convergence of CS and US-related information comes from single unit in vivo recordings obtained from the BLA. However, there are a number of limitations to single unit recordings. To address these issues, we employed whole-cell patch-clamp recordings from single BLA neurons in vivo and examined the convergence of auditory- and footshock-related inputs onto single BLA neurons. A glass electrode containing a standard potassium internal solution was lowered into the BLA of Wistar rats (P18-21) and a whole-cell recording configuration obtained. Using urethane-anesthetized animals we show that a single footshock (1ms, 5mA) can recapitulate UP-states while white noise (0.5-1s, 80 dB) only generates excitatory post-synaptic potentials (latency 100±11.2ms; duration 432±75 ms; amplitude: 10.43mV±1.70). Under isoflurane, the majority of BLA principal neurons displayed a depolarizing response to both white noise and footshock. The response-latencies were: white noise, 119.1 ± 6.3 ms; and footshock, 88.4 ± 3.6ms. These results demonstrate for the first time that single BLA projection neurons receive synaptic inputs related to both auditory and footshock stimulation.

## POS-TUE-117

# MAPPING THE PRIMATE ZONA INCERTA BY COMPARISON WITH THE PATTERN OF IMMUNOMARKERS IN THE RAT

Chipungu T.<sup>1</sup>, Thomas M.<sup>1,2</sup>, Lind C.<sup>2</sup> and Watson C.<sup>3</sup>

<sup>1</sup>Faculty of Computing Health and Science, Edith Cowan University.

<sup>2</sup>Department of Animal Biology, University of Western Australia.

<sup>3</sup>Health Sciences, Curtin University.

The zona incerta (ZI) has recently proved to be a more effective target than the subthalamic nucleus for deep brain stimulation (DBS) in patients with Parkinson's disease. The caudal ZI seems to be the most important area for DBS in humans, but the anatomy of the human ZI has not been mapped in detail. We have therefore attempted to identify the subnuclei of the primate ZI by comparison with the rat, in anticipation of extending the primate studies to the human brain in the future. We have examined serial brain sections stained with a number of markers (calbindin, parvalbumin, calretinin, SMI32, NADPH diaphorase, acetylcholinesterase, tyrosine hydroxylase, and Nissl) in the rat (n=1) (Paxinos et al 2009) and in the pygmy marmoset (n=1) (Tokuno et al, 2009). We have identified dorsal, ventral, rostral, and caudal regions of the ZI in the marmoset, which we believe to be homologous with similarly named areas in the rat. Parvalbumin, SMI32, and calretinin have proved the most useful markers in delineating sub-regions in the primate ZI. In the next phase of our study, we will extend the marmoset data to similarly stained sections of human ZI. Our long term aim is to correlate the area of optimal DBS in humans with an area with a characteristic pattern of histological markers.

## POS-TUE-119

# NETWORK ACTIVATION STATE MODULATES GAIN IN THE MOUSE LATERAL GENICULATE NUCLEUS

Wijesinghe R., Solomon S.G. and Camp A.J.

Discipline of Physiology and Bosch Institute, University of Sydney, Sydney, NSW 2006.

**Purpose.** Neurons in the visual thalamus receive synaptic inputs from a number of sources including the cerebral cortex, retina and brainstem nuclei. These inputs reflect the activation state of the network and interact with intrinsic membrane properties to determine individual neuronal output. We asked how the network activation state altered the output properties of neurons in the mouse (C57BL/6) lateral geniculate nucleus (LGN). **Methods.** Whole-cell current-clamp recordings were made from coronal brain slices (250 µm) containing the LGN. Intrinsic membrane properties of visualised LGN neurons were characterised using a set of stimuli that included incremental steps of depolarising current. Changes in network activation state were simulated by convolving a suite of white noise stimuli drawn from a Gaussian distribution (standard deviations; 6, 12, 25 or 50) onto the depolarising current stimuli. **Results.** LGN neurons showed a range of sensitivities to depolarising steps (firing response gain; 0.1-0.7 Hz/pA; n = 18). Increased noise altered firing response gain such that the distribution of gains in the LGN neuron population was narrowed. In 8/13 cells tested in this way, increased noise increased gain (p = 0.005), while in 5/13 neurons it decreased gain (p = 0.02). In both cases, changes were toward the population mean (0.32 ± 0.05 Hz/pA). However, pharmacologically altering the state of the corticothalamic network using the specific mGluR1a agonist 1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) always increased gain when compared to control (p = 0.016; n = 3). **Conclusion.** These results suggest the output of LGN neurons is stabilised around a mean level in a state-dependent manner, but presynaptic mGluR1a receptors on corticothalamic projecting neurons are not sufficient for this stabilisation.

## POS-TUE-118

# NEUROMODULATION OF STOCHASTIC RESONANCE IN CORTICAL NEURONS

Uusisaari M., Torben-Nielsen B. and Stiefel K.

OIST, 12-22 Suzaki, Uruma-shi, Okinawa-ken, 904-2234 Japan.

We investigated the properties of mouse cortical layer II/III pyramidal neurons to show stochastic resonance (SR), the property of signal transmission systems that allows them to function optimally under non-zero noise. Stochastic resonance was shown (N=10 cells) as the increase in signal to noise ratio with addition of noise to sub-threshold EPSPs injected via dynamic clamp. Depending on the intrinsic neuronal properties the optimal noise levels as well as the optimal timing of the inputs was found to differ. Even further, application of neuromodulatory agents such as carbachol modified the SR responses of neurons. As the ability of individual cell to respond and synchronize to subthreshold inputs greatly influences the large-scale behavior of the network, these results provide insights into the neuromodulation of synchronous activity of the cerebral cortex.

## POS-TUE-120

# ALCOHOL AND PROTEIN EXPRESSION: PERSPECTIVES ON ALCOHOL-INDUCED BRAIN DAMAGE

Kashem M.A. and McGregor I.S.

Psychopharmacology and Proteomics laboratory, School of Psychology, University of Sydney, NSW 2006, Australia.

**Background:** Alcohol can be harmful to the physical health, social life and brain of the individual. Alcohol-induced brain damage appears to be region-specific with major microstructural dysmorphology observed in the prefrontal cortex, the striatum, the cerebellum, the hippocampus and the white matter including the corpus callosum (CC). The molecular mechanisms underlying these microstructural changes in the human brain are largely unknown, particularly at the level of proteomics. **Methods:** Human postmortem brain samples were collected from NSW Tissue Resource Centre. Proteins were extracted from different brain regions (10 control; 7 uncomplicated alcoholic and 6 alcoholic complicated with hepatic cirrhosis) and separated by 2-D gel electrophoresis. Digitized gel images were analysed and identified protein through MALDI-TOF and the MASCOT search engine techniques. **Metabolic pathway analysis** of the identified proteins was performed using the Ingenuity database. **Results:** Four separate experiments were conducted using tissue from the hippocampus, and the genu, body and splenium of the corpus callosum. Differential expression of 21, 50, 46 and 43 proteins was found relative to controls in the alcoholic hippocampus, genu, body and splenium respectively. **Pathway analysis** suggested that differentially expressed proteins in these regions related to abnormal carbohydrate metabolism, lipid peroxidation, oxidative stress, signaling and apoptosis pathways. **Comparison** within alcoholic groups revealed that at least 40% of these proteins had differential expression in complicated (impaired liver function) compared to uncomplicated alcoholism. **Conclusion:** Sub-regional expression profiles indicate that alcohol-induced changes in protein expression are region specific. Liver complications have a synergistic effect on changes in brain protein expression. Thiamine-related cascades do not appear to be the major pathways for brain damage. Rather deleterious oxidative stress, methyl glyoxalation, lipid peroxidation, deacetylation and apoptosis pathways are dominant. Alcohol-induced microstructural damage detected histologically may therefore reflect cascades of these multiple biochemical mechanisms.



## POS-TUE-121

**LONG-TERM MODERATE BEER CONSUMPTION CAUSES MAJOR CHANGES IN THE STRIATAL PROTEOME OF THE RAT**

Kashem M.A., Sarker S., Ahmed E., **Ahmed S.**, Hargre G.A. and McGregor I.S.  
Psychopharmacology and Proteomics Laboratories, School of Psychology, University of Sydney, Brennan MacCallum Building, A18, NSW 2006, Australia.

**Background:** Dopamine systems centred on the ventral and dorsal striatum have been heavily implicated in the mechanisms underlying drug and alcohol abuse and addiction. Neuroimaging and other studies suggest that drug and/or alcohol addiction alters the neuronal plasticity in striatal regions through modification of neuronal structure and synaptic architecture. The biochemistry underlying those micro-morphological modifications induced by alcohol remains largely unknown. **Methods:** Group housed male Wistar rats given ad libitum home cage access to beer (Toohey's New) consumed alcohol at an average of 3.2 g/kg/d ethanol for 8 months. This represents a moderate level of alcohol consumption that is not associated with physical dependence. At the end of this drinking period, protein expression was studied in striatal tissue from these animals using 2-DE gel based proteomics. **Results:** Forty-four protein spots, recognised as 28 unique proteins, were differentially altered in the beer group relative to the controls ( $P < 0.05$ ). Functional analysis of the identified proteins indicated that they belonged to the general classes of metabolic (40%) followed by signal transduction (25%), oxidative stress (18%), cytoskeletal (10%) and Ca<sup>2+</sup> regulation (7%). Interestingly several dopamine (DA) regulating proteins such as tyrosine hydroxylase (enzyme of DA biosynthesis), pyridoxal phosphate phosphatase (coenzyme providing enzyme for DA biosynthesis), dopamine- and cAMP regulating phosphoprotein (DARPP-32) (protein of dopamine receptor and transporter regulator) and protein tyrosine phosphatase (DA signaling protein) and nitric oxide synthase (DA uptake modulating enzyme) were differentially expressed in the striatum. **Conclusion:** Prolonged moderate intake of alcohol can cause major changes in protein expression in the striatum of rats. The cascades of above identified proteins can be linked to altered synaptic plasticity and habitual alcohol seeking behaviour.

## POS-TUE-123

**ACUTE, REVERSIBLE AXONAL ENERGY FAILURE DURING A STROKE-LIKE EPISODE IN MELAS**

**Farrar M.A.**, Lin C.S.Y., Krishnan A., Park S., Andrews I. and Kiernan M.  
Prince of Wales Medical Research Institute, University of New South Wales.

**Introduction:** The pathophysiology of stroke-like episodes in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) remains unresolved. In-vitro studies of mitochondrial cytopathies have demonstrated reduced capacity to produce ATP during states of energy demand and membrane depolarization, resulting from Na<sup>+</sup>/K<sup>+</sup> pump inhibition. To date, in-vivo studies have largely focused on cerebral neuroimaging. **Methods:** Serial recordings of peripheral axonal excitability were undertaken at baseline and at 2, 4, 6, 24 and 48 hours from the onset of clinical symptoms during a stroke-like episode in a ten-year-old with MELAS. Serum electrolytes, lactate and pH were collected with each recording. **Results:** There were marked and progressive changes in multiple axonal excitability parameters during the first six hours of the stroke-like episode. The stimulus response curve shifted to the left, strength-duration time constant increased, threshold electrotonus 'fanned in', refractoriness increased and superexcitability reduced. These changes were consistent with axonal depolarization, similar to acute ischemia. There was a subsequent reversal of excitability parameters, with a return towards baseline by 24 hours. The excitability parameters correlated with the clinical assessment of CNS dysfunction and degree of lactic acidosis ( $R = 0.97$ ). **Conclusions:** There is dynamic and reversible depolarization in the peripheral axon due to disruption of energy dependent processes during a stroke-like episode in a 10-year-old with MELAS. **Significance:** Our results suggest that these pathophysiological processes may not be confined to the cortex, but rather may be deleterious to tissues with high energy demand, resulting in simultaneous energy insufficiency throughout the neural axis. As such, axonal excitability techniques may be useful as a surrogate marker of the central pathophysiological events that develop during a stroke-like episode in MELAS.

## POS-TUE-122

**IONIC HOMEOSTASIS IN RESPONSES OF NEURAL CELLS TO STRESS**

**Shabala L.**, Chung R.S. and West A.K.  
Menzies Research Institute, University of Tasmania.

It is well established that transport of ions is of paramount importance to neural cells and is believed to be among the first responses to chemical or physical injury. In our studies we assessed kinetics of net K<sup>+</sup>, Ca<sup>2+</sup> and H<sup>+</sup> fluxes in primary cortical neurons in response to a neurotransmitter glutamate thus simulating neural injury using a non-invasive microelectrode ion flux measuring technique (MIFE). All the chemical and physical injuries used led to a transient K<sup>+</sup> efflux and Ca<sup>2+</sup> uptake. Inhibitors of specific K<sup>+</sup> and Ca<sup>2+</sup> transport systems were used to assess their contribution to the observed ionic fluxes in response to stimuli thus revealing roles of relevant transporters in stress perception and signalling. Application of various concentrations of glutamate (between 1 to 100  $\mu$ M) led to a transient K<sup>+</sup> efflux and Ca<sup>2+</sup> and H<sup>+</sup> uptake with the magnitude of response being concentration dependent. Peak of K<sup>+</sup> efflux increased from 184.63 nmol m<sup>-2</sup> s<sup>-1</sup> to 1814.42 nmol m<sup>-2</sup> s<sup>-1</sup>, respectively, when glutamate concentration was raised from 1 to 100  $\mu$ M ( $n = 3-4$ ). The magnitudes of fluxes were age specific with 3-fold increase in Ca<sup>2+</sup> uptake in 14 DIV as compared to 7 DIV neurons, while K<sup>+</sup> efflux measured from the same cells was decreased in mature cultures. Inhibitors of ionotropic glutamate receptors MK-801 and CNQX significantly reduced net Ca<sup>2+</sup> influx suggesting their involvement in the observed flux changes. TEA and 4-AP reduced K<sup>+</sup> efflux by 50% and 30%, respectively ( $n = 3-5$ ), suggesting involvement of more routes for K<sup>+</sup> efflux. Overall, our results demonstrate that studies of kinetics of ion transport across cellular membranes can help to understand better mechanisms underlying those processes.

## POS-TUE-124

**PATHOPHYSIOLOGY OF PACLITAXEL-INDUCED NEUROTOXICITY**

**Park S.B.**, Lin C.S.Y., Krishnan A.V., Friedlander M.L., Lewis C. and Kiernan M.C.  
Prince of Wales Medical Research Institute and Clinical School, University of New South Wales.

**Purpose** Sensory neurotoxicity is a prominent side-effect of the chemotherapy paclitaxel, commonly utilised in early-stage breast cancer. Although *in-vitro* studies suggest that disruption of axoplasmic transport may underlie neuropathy development, pathophysiology remains largely undefined. The present study aims to investigate the development of paclitaxel-induced neurotoxicity *in-vivo* to explore pathophysiological mechanisms. **Method** Sensory axonal excitability studies, quantitative sensory testing and clinical neurotoxicity grading scales were undertaken in 15 paclitaxel-treated patients (126 studies), across assessed prospectively at baseline and every month during treatment. A cohort of 17 oxaliplatin-treated patients (590 studies) was included as a disease control. **Results** Following 930 $\pm$ 64.3 mg/m<sup>2</sup> paclitaxel over 11.5 weeks, 60% of patients reported neuropathic symptoms, which developed at a median of 6 weeks of treatment. Stimulus threshold significantly increased ( $P < 0.05$ ) after 4 weeks, while by mid-treatment, maximal sensory amplitude was significantly reduced from 50.1 $\pm$ 4.7  $\mu$ V to 41.2 $\pm$ 4.0  $\mu$ V ( $P < 0.05$ ). By final treatment, sensory amplitude was reduced to 36.5 $\pm$ 3.3  $\mu$ V ( $P < 0.05$ ), revealing development of sensory nerve damage. However, excitability measures of membrane potential and ion channel function remained within normal limits. In contrast, 11 weeks of oxaliplatin treatment produced no reduction in maximal sensory amplitude but significant excitability changes revealing membrane hyperpolarization ( $P < 0.05$ ). **Conclusion** Chemotherapy-induced neurotoxicity is associated with a diverse range of excitability profiles reflecting differing underlying pathophysiologicals. Specifically, paclitaxel treatment produces progressive changes in sensory nerve function, in the absence of alterations in membrane potential or ion channel function. In contrast, oxaliplatin causes significant changes in excitability suggestive of membrane hyperpolarization that precedes axonal degeneration. These findings suggest that excitability studies may provide novel biomarkers of axonal function in chemotherapy-induced neurotoxicity.

## POS-TUE-125

## HCN CHANNELS IN INFLAMMATION OF THE RAT TEMPOROMANDIBULAR JOINT

Hatch R.J., Staikopoulos V., Ivanusic J.J. and Jennings E.A.  
Department of Anatomy and Cell Biology, University of Melbourne,  
VIC 3010.

**Introduction:** Hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels are active at resting membrane potentials and thus contribute to neuronal excitability. Four HCN subunits have been cloned (HCN1-4) and have been shown to contribute to nociception following nerve injury. However, little is known of the contribution of HCN channels to nociception following inflammation. **Methods:** In one series of experiments, an animal model of sensitivity to temporomandibular joint (TMJ) inflammation was used to determine whether peripheral HCN channels contribute to inflammatory pain. Sensitivity was tested with calibrated von Frey filaments, applied over the TMJ, 1 day pre- and 1 day post-injection of 4µl of complete Freund's Adjuvant (CFA; to induce inflammation; n=7 animals), saline (control; n=7 animals) or CFA+ZD7288 (inflammatory agent+HCN antagonist; n=8 animals). Withdrawal thresholds at day 1 post-injection were determined and expressed as a percentage of the pre-injection value for each animal. In another series of experiments (n=3 animals), retrograde tracing was used to identify trigeminal sensory neurons that innervate the TMJ, and immunohistochemistry was used to examine HCN1-4 subunit immunoreactivity in these neurons. **Results:** There was a significant increase in mechanical sensitivity (decrease in withdrawal threshold) in animals following CFA injection, but not following saline injection, and ZD7288 blocked this increase in sensitivity (ANOVA on ranks;  $P < 0.05$ ). Of the neurons retrogradely labelled with Fast Blue,  $13 \pm 1.7\%$  (mean  $\pm$  SEM),  $13 \pm 4.6\%$ ,  $1.4 \pm 0.8\%$  and  $0\%$  were immunoreactive for the HCN1, HCN2, HCN3 and HCN4 subunits respectively. **Conclusion:** These data suggest that HCN channels on trigeminal primary afferent neurons contribute to nociception induced by inflammation.

## POS-TUE-127

## ELECTROPHYSIOLOGICAL PROPERTIES OF SUPERFICIAL DORSAL HORN NEURONS DIFFER IN UPPER-CERVICAL SPINAL SEGMENTS IN NEONATE AND ADULT MICE

Harris B.M., Walsh M.A., Graham B.A., Bolton P.S., Brichta A.M. and Callister R.J.  
Biomedical Sciences & Pharmacy, University of Newcastle,  
Callaghan, NSW 2308.

Injury to deep structures in the neck produces significant disability, including ongoing pain. Such injuries often occur in neonates because of torsional forces associated with forceps delivery, and in adults following whiplash. Surprisingly, little is known about how sensory information from deep neck structures is processed in upper-cervical spinal segments in neonates or adults. **Purpose:** To investigate excitability and sensory processing in upper-cervical (C2-4) superficial dorsal horn (SDH) neurons in neonate (P0-5) and adult ( $\geq$  P24) mice. **Methods:** Mice (C57Bl/6) were anaesthetized (Ketamine 100 mg/kg i.p.) and decapitated. Transverse slices were prepared from C2-4 spinal segments and whole cell recordings (at  $32^\circ\text{C}$ ) were obtained from SDH neurons ( $\text{KCH}_3\text{SO}_4$  internal). **Results:** Passive membrane properties differed significantly between neonatal (n=101) and adult (n=99) SDH neurons. Input resistance was higher in neonates ( $711 \pm 41 \text{ M}\Omega$  vs.  $428 \pm 21 \text{ M}\Omega$ ) and resting membrane potential was more depolarised ( $-58.4 \pm 1.0 \text{ mV}$  vs.  $-65.8 \pm 1.0 \text{ mV}$ ). Action potential (AP) and after-hyperpolarization amplitude were lower in neonates ( $29.5 \pm 1.4 \text{ mV}$  vs.  $48.9 \pm 1.3 \text{ mV}$ ;  $-11.5 \pm 1.0 \text{ mV}$  vs.  $-36.9 \pm 0.7 \text{ mV}$ ), whereas AP half-width was greater in neonates ( $2.29 \pm 0.1 \text{ ms}$  vs.  $0.72 \pm 0.02 \text{ ms}$ ). Neonate SDH neurons also differed in their response to step current injection. The prevalence of tonic firing and initial bursting responses was greater in adult neurons. Following dorsal root stimulation (C2 nerve) both A $\delta$  and C-fibre inputs were observed in adult SDH neurons. **Conclusion:** Our data suggest nociceptive processing in the upper-cervical SDH differs in neonates and adults.

## POS-TUE-126

## DISTINCT MEMBRANE AND SYNAPTIC PROPERTIES OF CALRETININ EXPRESSING NEURONS IN THE SUPERFICIAL DORSAL HORN OF THE MOUSE SPINAL CORD

Graham B.A.<sup>1</sup>, Hughes D.I.<sup>2</sup>, Lim R.<sup>1</sup>, Sah P.<sup>3</sup>, Brichta A.M.<sup>1</sup> and Callister R.J.<sup>1</sup>

<sup>1</sup>University of Newcastle and HMRI, Newcastle. <sup>2</sup>University of Glasgow, Scotland. <sup>3</sup>University of Queensland and QBI, Brisbane.

Neurons in the superficial dorsal horn (SDH) receive and process noxious and innocuous peripheral inputs. One barrier to understanding how SDH neurons process such inputs has been the regions neuronal heterogeneity. **Purpose:** We used transgenic mice, expressing enhanced green fluorescent protein (eGFP) in calretinin-positive neurons, to record selectively from presumptive excitatory interneurons. **Methods:** Mice (2-3 months) were anaesthetized (Ketamine 100 mg/kg i.p.), decapitated, and transverse slices were prepared from lumbar spinal cord. Targeted patch-clamp recordings were made from eGFP-positive neurons and compared to those from randomly sampled neurons in littermate controls. **Results:** eGFP neurons (n = 30) had similar input resistances ( $345 \pm 27 \text{ M}\Omega$  vs.  $364 \pm 43 \text{ M}\Omega$ ) and RMPs ( $-63 \pm 1 \text{ mV}$  vs.  $-63 \pm 2.5 \text{ mV}$ ) to randomly sampled neurons (n = 18), however membrane capacitance was increased ( $25 \pm 1 \text{ pF}$  vs.  $20 \pm 2 \text{ pF}$ ,  $p < 0.05$ ). Spontaneous excitatory postsynaptic currents (sEPSC) in eGFP neurons (n = 20) had similar amplitudes ( $-32.2 \pm 12.1 \text{ pA}$  vs.  $-28.5 \pm 1.8 \text{ pA}$ ), rise times ( $0.55 \pm 0.02 \text{ ms}$  vs.  $0.60 \pm 0.03 \text{ ms}$ ) and half widths ( $1.7 \pm 0.1 \text{ ms}$  vs.  $1.9 \pm 0.1 \text{ ms}$ ) to randomly sampled neurons (n = 18), however sEPSC frequency was higher ( $29.0 \pm 1.0 \text{ Hz}$  vs.  $16.6 \pm 1.9 \text{ Hz}$ ,  $p < 0.05$ ). The rapidly activating and inactivating potassium current (I<sub>Ar</sub>) was observed in all eGFP neurons (18/18) but only 11/17 of randomly sampled recordings. **Conclusions:** The distinct properties of calretinin expressing eGFP-positive neurons suggest a specific role for these presumptive excitatory interneurons in the spinal sensory processing.

## POS-TUE-128

## COMPARISON BETWEEN T-LYMPHOCYTES IN DORSAL ROOT GANGLIA OF WISTAR AND LEWIS RATS AFTER SCIATIC NERVE TRANSECTION

Hu P.<sup>1</sup> and McLachlan E.M.<sup>1,2</sup>

<sup>1</sup>Prince of Wales Medical Research Institute, Randwick, NSW 2031.

<sup>2</sup>University of New South Wales, Sydney, NSW 2052.

T-lymphocyte invasion of dorsal root ganglia (DRGs) projecting in damaged peripheral nerves may arise from an auto-immune response [1] and be involved in retrograde death of axotomized neurones. Here we compared T-cell responses to nerve transection between Wistar and Lewis rats. The latter (with few T-cells and heightened susceptibility to autoimmune disease) might respond more than Wistar rats. The left sciatic nerve was transected in groups of 6 female rats (7-9 weeks old) under anaesthesia with ketamine (60 mg/kg) and xylazine (10 mg/kg) i.p. After one or 10 weeks, the rats were anaesthetized with pentobarbitone (100 mg/kg i.p.) and perfused with Zamboni's fixative. Bilateral L5 DRGs were processed for double labelling immunohistochemistry, using combinations of antibodies to  $\alpha/\beta$  T-Cell Receptor, CD8, CD3, CD68 and MHC II to define the subtypes of T-cells and macrophages. After one week, T-cell density was higher in Wistar than Lewis rats and, after 10 weeks, was markedly increased in both strains. However the increase relative to that after one week was similar between strains. The data suggested that CD4+ T-cells are more prevalent after injury in Lewis than Wistar rats. Increased MHC II+ macrophage density paralleled that of T-cells in both strains. As T-cells can be beneficial or detrimental for survival of axotomized adult retinal ganglion cells [2], the degeneration of cutaneous nociceptive neurones after peripheral nerve injury [3] needs evaluation in both strains. 1 Olsson, T. et al (1992) Autoimmunity 13:117-126. 2. Luo, J-M. et al (2007) Eur. J. Neurosci. 26:3475-3485. 3 Hu, P. & McLachlan, E.M. (2003) J. Neurosci. 23:10559-10567.

## POS-TUE-129

**LONG-TERM FACILITATION OF SYMPATHETIC NERVE ACTIVITY DOES NOT REQUIRE INCREASED PHRENIC NERVE ACTIVITY FOLLOWING ACUTE INTERMITTENT**

Xing T., Sun Q., McMullan S. and Pilowsky P.  
Australian School of Advanced Medicine, Macquarie University.

Intermittent hypoxia (IH) elicits a long-lasting augmentation of phrenic nerve activity known as long-term facilitation (LTF), a type of plasticity of respiratory motor neural activity, even after the stimuli have ceased and blood gases have normalized. It is still unclear whether the sympathetic system similarly expresses the IH-induced plasticity, even though the respiratory and sympathetic control systems are coupled with each other. The aim of this study was to investigate the relationship between the sympathetic and phrenic LTF after IH. We recorded splanchnic (sSNA) and phrenic nerve activities (PNA) in urethane-anaesthetized (1.2 g/kg, i.p.), vagotomized and mechanically ventilated Sprague-Dawley rats (n=16). Animals (n=11) were exposed to 10 45s episodes of 10% O<sub>2</sub>-90% N<sub>2</sub>, separated by 5 min interval of 100% O<sub>2</sub>, and the recordings were continued for 60 min following the last hypoxic exposure. The other five animals were prepared in the same manner but not exposed to hypoxia (time-control). Cycle-triggered averages of integrated PNA and sSNA from periods preceding, and 15, 30, 45 and 60 min following the hypoxic stimuli were compared. We found that (1) all animals manifested the sustained increase of sSNA (P<0.001) after AIH, but only five of them also expressed phrenic LTF compared with the time control group; (2) both the inspiratory, and post-inspiratory, peaks of SNA increased regardless of phrenic LTF; (3) the baroreflex was enhanced after the sympathetic LTF was established (Gain<sub>max</sub> from 2.15±0.37 to 3.62±0.98 %/mmHg, P=0.008). These findings indicate that the respiratory-sympathetic coupling does contribute to sympathetic LTF, but that an additional effect on sympathetic tone is also present.

## POS-TUE-131

**DEVELOPMENTAL CHANGES IN SUBTHRESHOLD POTASSIUM CURRENTS CONTRIBUTE TO INCREASED EXCITABILITY OF THE NEONATAL SPINAL CORD**

Walsh M.A., Graham B.A., Brichta A.M. and Callister R.J.  
Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW, 2308.

There is now increasing evidence that innocuous and noxious peripheral stimuli are processed differently in the spinal cords of neonates and adults. Spinal superficial dorsal horn (SDH) neurons receive and process these stimuli, and their excitability plays an important role in determining SDH output to higher centres. Subthreshold potassium (K<sup>+</sup>) currents are important in setting excitability and discharge in a variety of CNS neurons. It is not known how these K<sup>+</sup> currents differ in neonate and adult SDH neurons. **Purpose:** To compare the properties of subthreshold K<sup>+</sup> currents in neonatal (P0-5) and adult (≥ P21) SDH neurons. **Methods:** Mice were anaesthetized (Ketamine 100 mg/kg i.p.), decapitated and transverse slices (300 µm, L3-5 segments) were prepared. Whole cell recordings were obtained (at 32°C) from SDH neurons using a KCH<sub>3</sub>SO<sub>4</sub>-based internal. **Results:** Two distinct outward currents were observed in adult neurons, one exhibiting rapid activation/inactivation (rapid A; I<sub>Ar</sub>) and a second with slower kinetics (slow A; I<sub>As</sub>). Only I<sub>Ar</sub> was observed in neonatal SDH neurons, so further comparisons were restricted to I<sub>Ar</sub>. The peak amplitude (158.97 ± 13.97 pA vs. 346.28 ± 38.99 pA; neonates (n = 80) vs. adults (n = 59)) and decay time constant (26.3 ± 26 ms vs. 38.8 ± 3.5 ms) of I<sub>Ar</sub> was lower in neonates. Further analysis of I<sub>Ar</sub> (n=10; both ages) revealed neonatal I<sub>Ar</sub> activated at more depolarised (~5 mV) potentials. Inactivation kinetics did not differ in neonates and adults. **Conclusion:** The lower expression, faster kinetics and more depolarised activation threshold of I<sub>Ar</sub> are consistent with the increased excitability of the neonatal spinal cord.

## POS-TUE-130

**NERVE EXCITABILITY AND MECHANISMS OF IMMUNOTHERAPY IN PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY**

Lin C.S.-Y.<sup>1,3</sup>, Krishnan A.V.<sup>1,3</sup>, Park S.B.<sup>2,3</sup> and Kiernan M.C.<sup>2,3</sup>  
<sup>1</sup>School of Medical Sciences, UNSW. <sup>2</sup>Prince of Wales Clinical School, UNSW. <sup>3</sup>Prince of Wales Medical Research Institute.

Purpose Chronic inflammatory demyelinating polyneuropathy (CIDP) is a disorder of the peripheral nervous system treated using immunotherapy intravenous immunoglobulin (IVIg). However, although IVIg is the first-line treatment for CIDP, the underlying mechanism of IVIg remains unclear. To investigate the pathophysiology of immunotherapy, nerve excitability studies were undertaken to monitor changes reflected in axonal membrane potential during treatment. Method Motor nerve excitability studies were undertaken in 20 patients undergoing immunotherapy (IVIg), pre-and-post monthly infusion. In addition, CIDP patients were assessed longitudinally across treatments. The median nerve was stimulated at the wrist, recording compound motor action potentials (CMAP) from abductor pollicis brevis muscle. Multiple excitability parameters were recorded including stimulus-response curves (SR), threshold electrotonus (TE), recovery cycle of excitability (RC) and current-voltage relationship (I/V). Results Patients with CIDP demonstrated significant differences (paired t-test) in multiple excitability parameters pre and post infusion. Post-infusion, the stimulus response curve shifted to the left (decreased threshold) with increased peak response (p<0.05). SDTC was significantly reduced (p<0.02) accompanied with fanning-in appearance of TE (TE<sub>H</sub>(90-100)ms, p<0.01) and increase refractoriness and decreased superexcitability in the RC. Longitudinal changes in these excitability parameters, specifically superexcitability demonstrated correlation with the clinical recovery of patients. Conclusion This is the first study using novel threshold tracking techniques to investigate the mechanisms of action of immunotherapy in CIDP patients. Significant changes in excitability properties illustrated a subtle depolarizing effect post-immunotherapy. These results may imply the role of immunotherapy is to stabilize the axonal membrane potential to thereby prevent further progression due to the underlying disease state.

## POS-TUE-132

**SODIUM CURRENT PROPERTIES DIFFER IN NEONATE AND ADULT MOUSE SUPERFICIAL DORSAL HORN NEURONS**

Farrell K.E., Walsh M.A., Graham B.A., Brichta A.M. and Callister R.J.  
Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW, 2308.

Superficial dorsal horn (SDH: laminae I-II) neurons are important for spinal processing of nociceptive information and their excitability is determined, in part, by the properties of voltage-gated sodium channels (I<sub>Na</sub>). Recently, we have shown excitability and action potential (AP) properties in SDH neurons are altered during development (Walsh et al., 2009 J Neurophysiol 101: 1800). **Purpose:** To compare I<sub>Na</sub> currents in neonate (P0-5) and adult (≥ P21) SDH neurons. **Methods:** Mice were anaesthetized (Ketamine 100 mg/kg i.p.) and decapitated. Transverse slices were prepared from L3-5 spinal segments and whole-cell recordings were made (at 32°C) from visualized SDH neurons using a CsF-based internal. **Results:** A fast activating and inactivating inward current was evoked by depolarising neurons from -60 to -20 mV. The peak amplitude of this current increased during development (1.34 ± 0.35 nA vs. 6.58 ± 0.68 nA; neonates (n = 10) vs. adults (n = 12)), and was completely abolished by 1 µM TTX in both neonates (n = 2) and adults (n = 3), thus confirming the current was mediated by I<sub>Na</sub>. Time to peak and half width was slower in neonates (0.82 ± 0.08 ms vs. 0.46 ± 0.06 ms; 1.40 ± 0.25 ms vs. 0.43 ± 0.05 ms). I<sub>Na</sub> activation voltage and peak current voltage also differed in neonatal and adult neurons (-50 mV vs. -60 mV; -15 mV vs. -25 mV). **Conclusion:** These data show I<sub>Na</sub> expression and kinetics differ in neonate and adult SDH neurons and provide an underlying mechanism for the differences observed previously in AP properties of developing SDH neurons.



## POS-TUE-133

# REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION CHANGES AN ABNORMAL - BUT NOT NORMAL - NEURAL PROJECTION

Mo C.<sup>1</sup>, Sherrard R.<sup>2</sup>, Dunlop S.<sup>1</sup> and Rodger J.<sup>1</sup>

<sup>1</sup>School of Animal Biology, The University of Western Australia.

<sup>2</sup>School of Anatomy and Human Biology, The University of Western Australia, Crawley WA.

**Purpose:** Non-invasive and painless stimulation of brain tissue by administration of repetitive transcranial magnetic stimulation (rTMS) benefits a wide range of neurological and psychiatric disorders. However, investigations have been limited to synaptic changes in healthy animals, short-term effects which are unlikely to explain long-lasting behavioural improvements in neurological conditions. Here, we investigate the potential for rTMS to alter connectivity in a representative neural projection, the mouse retinocollicular projection. In addition, we compared the effect of rTMS on a normal (wildtype) and abnormal (ephrin-A2/A5-/- mice) projection. **Methods:** After 14 days (10min/day) of high frequency (>75Hz) stimulation to the superior colliculus, or sham treatment, the retinocollicular projection was assessed anatomically (anterograde tracing) and functionally (electrophysiological recording, visuomotor behaviour) in WT (n=40) and ephrin-A2/A5-/- (n=19) mice. Data were analysed using ANOVA and Scheffe post-hoc tests. **Results:** rTMS altered functional properties of the retinocollicular projection in ephrin-A2/A5-/- but not WT mice. Sham-treated ephrin-A2/A5-/- mice showed a longer latency of response to off-light stimulation, and this was reduced by rTMS treatment to the same as WT (p<0.05). In addition, rTMS increased the size of the retinocollicular receptive fields (p<0.0001 vs sham). No anatomical or behavioural changes were detected following rTMS. **Conclusion:** Chronic, high frequency rTMS can induce subtle functional changes in a mature neural projection. However, these changes were only observed in a neural system with abnormal connectivity (ephrin-A2/A5-/- mice), suggesting homeostatic mechanisms may prevent such changes in normal mice. Our results highlight the importance of using relevant animal models of neurological disorders in investigating the impact of rTMS.

## POS-TUE-135

# DOPAMINE D2 RECEPTOR EXPRESSING STRIATAL PROJECTION NEURONS DISPLAY LONG TERM POTENTIATION AFTER HIGH FREQUENCY STIMULATION OF CORTICAL AFFERENTS

Vickers C.A., Arbuthnott G.W. and Wickens J.

Neurobiology Research Unit, Okinawa Institute of Science and Technology, Uruma, Okinawa, Japan.

Activity-dependent synaptic plasticity in the neostriatum has been proposed to play an important role in the integration of cortical information into specific reward related actions. Experimental analysis of plasticity in this system is complicated by the existence of two major subtypes of striatal projection neurons, which predominantly express either dopamine D1 receptors (D1 cells) or D2 receptors (D2 cells). Here, we study synaptic plasticity in corticostriatal slices from transgenic mice that have cell specific markers to allow definitive identification of D1 and D2 cells. After high frequency cortical stimulation (100 Hz), D1 and D2 cells displayed cell specific responses. The D2 cells potentiated to a significantly higher degree than the D1 cells, suggesting that afferent information integration and processing is different in the two cell types. The potentiation observed in the D2 cells was sensitive to the specific D2 receptor antagonist sulpiride (10µM) and the adenosine A2A receptor antagonist ZM241385 (1µM). Analysis of the cells' electrophysiological properties revealed D2 cells were more excitable than D1 cells. Application of the A2A antagonist modulated the pattern of firing of the D2 cells so that they resembled those seen in the D1 cells. These data suggest that there are specific differences in synaptic plasticity between D1 and D2 cells in response to specific patterns of excitation and that there is a significant role for both the dopamine D2 receptor and adenosine A2A receptor in modulating the plasticity in D2 cells.

## POS-TUE-134

# LONG-TERM DEPRESSION CAUSED BY LOW FREQUENCY STIMULATION IN PAIRED-PYRAMIDAL CELLS OF LAYER II/III OF RAT BARREL CORTEX

Li L.<sup>1,3</sup>, Choy J.<sup>1</sup> and Stricker C.<sup>1,2</sup>

<sup>1</sup>The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200. <sup>2</sup>ANU Medical School, The Australian National University, Canberra, ACT 0200. <sup>3</sup>School of Medicine, Xi'an Jiaotong University, Shaanxi, China, 710061.

Multiple forms of long-term depression (LTD) have been reported at neocortical synapses, implying their varied roles in different forms of behaviour. Using low frequency presynaptic stimulation (<0.3 Hz) in paired-recordings, we observed LTD with unique properties. **Purpose:** Identifying the induction site and exploring underlying molecular mechanism(s) of this LTD. **Methods:** Paired whole-cell recording from layer II/III pyramidal cells, verified by histology, were obtained in 300 µm thick slices of barrel cortex (P15-19) at 36±1°C. Pre- and postsynaptic cells were current- and voltage-clamped, respectively. 300 presynaptic stimuli were evoked at 0.2 Hz using short current pulses (~1 nA, 3 ms) in the presence of gabazine (3 µM). **Results:** Repeated stimulation caused LTD of 69% after 25 min, lasting >2.5 h. Rate of depression was well fit by a single exponential (tau=25.5±3.9 min at 0.25 Hz, n=9) and still observed at 0.03 Hz (tau=121±29 min, n=5). tau was shorter for paired versus single stimuli at 0.1 Hz (25±7 vs 109±28 min, n=3). Paired-pulse interval did not affect tau. Chelation of postsynaptic Ca<sup>2+</sup> with intracellular BAPTA (20 mM), did not prevent LTD, indicating a presynaptic mechanism. Blockade of presynaptic adenosine-1, NMDA and P2X/Y receptors by DPCPX (200 nM), APV (20 µM) and suramin (20 µM), respectively, had no effect. **Conclusions:** A presynaptic form of LTD is described, lasting > 2.5 h in layer II/III pyramids of barrel cortex, which is independent of presynaptic A<sub>1</sub>, NMDA or P2X/Y receptor activation. CB1R and mGluR are currently being explored.

## POS-TUE-136

# PROTEIN SYNTHESIS-DEPENDENT ENHANCEMENT OF TRANSMITTER RELEASE IN PERSISTENT FORMS OF HIPPOCAMPAL LTP

Johnstone V.P.A. and Raymond C.R.

Neuroscience Program, The John Curtin School of Medical Research and The Eccles Institute of Neuroscience, The Australian National University, Canberra, ACT.

Long-term potentiation (LTP) of synaptic transmission is an important process underlying learning and memory in the brain. At CA3-CA1 synapses in the hippocampus, three discrete forms of LTP (LTP1, 2 and 3) can be differentiated on the basis of maintenance and induction mechanisms. However, the relative roles of pre- and postsynaptic expression mechanisms in LTP1, 2 and 3 are unknown. In this study, the potential role of enhanced neurotransmitter release in the expression of LTP1, 2 and 3 was investigated by measuring electrically-evoked destaining of the styryl dye FM1-43 from potentiated CA3 terminals in 400µm brain slices taken from male Wistar rats (7-8 weeks). No difference in vesicle turnover rate was observed for LTP1 at 60 min or 120 min following induction by 1 train of theta-burst stimulation (1TBS). A significant increase in release was found for LTP2 only at 120 min after induction by 4TBS (n=6; p<0.05), and for LTP3 at both time points after induction by 8TBS (60 min, n=14; 120 min, n=10; p<0.05). Inhibition of protein synthesis with anisomycin blocked both LTP2 maintenance (n=4, p<0.05) and the associated enhanced exocytosis (n=3, p<0.05), whereas the transcription inhibitor Actinomycin-D had no effect. LTP3 was found to be dependent on both protein synthesis (n=4, p<0.05) and transcription (n=6, p<0.05), however the associated enhanced release was dependent only on protein synthesis (n=4, p<0.05). This study shows that more durable forms of LTP involve an enhancement of transmitter release, which is dependent on *de novo* protein synthesis, but not gene transcription, confirming the existence of mechanistically discrete forms of LTP in CA1.

## POS-TUE-137

**CHEMOKINE-MEDIATED GUIDANCE OF THE NEUROINFLAMMATORY RESPONSE BY MÜLLER CELLS FOLLOWING LIGHT-INDUCED RETINAL DEGENERATION**Rutar M.<sup>1,2</sup>, Natoli R.<sup>1,2</sup>, Valter K.<sup>1,2</sup> and Provis J.M.<sup>1,2</sup><sup>1</sup>Research School of Biology, ANU, ACT, 2601. <sup>2</sup>ARC Centre of Excellence in Vision Science, ANU, ACT, 2601.

**AIM:** To investigate the role of the chemokine CCL-2 – a monocyte chemoattractant protein – in shaping the retinal inflammatory response following photoreceptor degeneration induced through exposure to excessive light. **METHODS:** SD rats were exposed to 1000lx of light for up to 24hrs, after which some animals were kept in dim light (5 lux) to recover. At specific time points during (1, 3, 6, 12, 17, and 24hrs) and following exposure (3 and 7 days), animals were euthanized and retinas processed. CCL-2 expression was assessed by qPCR (n=4), immunohistochemistry (n=3), and *in situ* hybridization (n=3) at each time point. In conjunction, counts were made of monocytes on retinal cryo-sections immunolabeled with ED1 (n=4), while photoreceptor cell apoptosis was assessed using TUNEL labeling (n=5). Statistical significance was determined using the Students t-test. **RESULTS:** Up-regulation of CCL-2 gene expression was evident in retinal tissue after 12hrs exposure, which correlated with the significant increase (p<0.05) in photoreceptor cell death. CCL-2 expression peaked by 24hrs exposure, coinciding with the peak in cell death. Immunohistochemistry and *in situ* hybridization on retinal cryo-sections revealed that CCL-2 is expressed by Müller cells from 12hrs exposure onward, predominately in regions of heavy photoreceptor degeneration. From 24hrs exposure, a significant (p<0.05) recruitment of monocytes to the choroidal and retinal vascular supplies was observed. CCL-2 immunoreactivity was also observed in many of these infiltrating monocytes at 24hrs exposure. **CONCLUSION:** Our data indicate that photoreceptor death promotes CCL-2 expression by Müller cells, which facilitates the targeting of monocytes to sites of injury, thereby contributing to the guidance of the neuroinflammatory response following retinal injury.

## POS-TUE-139

**ANTI-INFLAMMATORY EFFECT OF 670NM LIGHT IN WHITE LIGHT-INDUCED PHOTORECEPTOR DEGENERATION**Albarracin R.S.<sup>1,2</sup> and Valter K.<sup>1,2</sup><sup>1</sup>Research School of Biology, The Australian National University, Canberra, Australia. <sup>2</sup>ARC Centre of Excellence in Vision Science, ANU, Canberra, Australia.

**Purpose:** The aim of this study is to assess the long term effect of 670nm red light in modulating inflammatory response in retinas damaged by exposure to white light. **Methods:** Young SD rats were exposed to bright continuous light (BCL) for 24 hours. Animals were divided into 3 groups (n=8 per group). The first group was treated with 670nm red light (NIR) at 10J/cm<sup>2</sup> using an LED array 1x daily for 5 days prior to light exposure (pre-treatment). The second group was treated immediately after the cessation of BCL for 5 days (post-treatment). The third group was treated 1 day prior to BCL, then 2x daily during and immediately after BCL (mid-treatment). Retinal function was evaluated 1week and 1month after light exposure using ERG. Cell damage was assessed using classical histology and TUNEL. Immunohistochemistry, Western Blot and qPCR were performed to assess localisation and regulation of activated microglia and complement components. **Results:** Population of photoreceptors was maintained in all NIR treated groups compared to the non-treated animals (p<0.01). The photoreceptor function was only reduced by 10-30% in pre- and mid-treatments groups compared to the 80-90% loss in non-treated retinas (p<0.01). The post treatment group showed functional damage at 1week but recovered to 80% of baseline by 1month (p<0.01). The invasion of activated microglia/macrophages was prominent in non-treated but was not observed in the NIR treated retinas. Complement activation was also reduced in treated animals compared to non-treated groups. **Conclusions:** Present results suggest that NIR treatment may ameliorate the effect of damaging light thereby providing protection and long term stability of the retina against light-induced degeneration.

## POS-TUE-138

**ROLE OF THE COMPLEMENT SYSTEM IN ACUTE AND CHRONIC MODELS OF RETINAL DEGENERATION**Provis J.M.<sup>1,2</sup>, Rutar M.<sup>1,2</sup>, Natoli R.<sup>1,2</sup> and Valter K.<sup>1,2</sup><sup>1</sup>Research School of Biology, ANU, ACT, 2601. <sup>2</sup>ARC Centre of Excellence in Vision Science, ANU, ACT, 2601.

**AIM:** To investigate the role of the complement system in the pathogenesis of retinal disease by assessing the expression profile of key complement components in two distinct rodent models: acute degeneration induced through exposure to excessive light, and chronically using a degenerative rodent strain (P23H). **METHODS:** In the acute model, SD rats were exposed to 1000lx of light for up to 24hrs, after which some animals were kept in dim-light (5 lux) to recover. At specific time points during and following exposure, animals were euthanized and retinas processed. In the chronic model, degenerative P23H rats and non-degenerative SD rats of similar ages were euthanized and retinas processed for comparative analysis. The expression of complement component genes (C1s, C3, and C5), complement receptor genes (C1qR, C3aR, C5aR), and a retinal stress gene (GFAP) were assessed by qPCR (n=3). Photoreceptor cell apoptosis was determined at each time point (n=5) using TUNEL labeling. Statistical significance was assessed using the One-way ANOVA. **RESULTS:** A significant up-regulation (p<0.0001) of C3, C1s, C3aR, C1qR, and C5aR was observed during and following the course of light exposure, correlating with significant increases in photoreceptor apoptosis (p<0.001) and GFAP up-regulation (p<0.0001). Comparison between SD and P23H rats showed modest increases in C3 and C1s expression in the P23H strain, consistent with the slow, persistent degeneration characterized in this model. **CONCLUSION:** While the degenerative stimuli in both models differ, the increased expression of key complement components – which fuel the complement cascade – in conjunction with increasing photoreceptor death provides evidence for a common pathway in retinal degeneration involving the activation of complement.

## POS-TUE-140

**RATIONALE FOR NON-INVASIVE TREATMENT OF ROP: DARK REARING MINIMISES VASO-OBLITERATION DURING HYPEROXIA AND MIMICS PHYSIOLOGICAL VASCULARIZATION**Chan-Ling T.<sup>1</sup>, Natoli R.<sup>2</sup>, Provis J.<sup>2</sup>, Bisti S.<sup>3</sup>, Maccarone R.<sup>3</sup> and Yun S.<sup>1</sup><sup>1</sup>Department of Anatomy, Bosch Institute, University of Sydney.<sup>2</sup>Research School of Biology, Australian National University. <sup>3</sup>Department of Biomedical Science and Technology, University of L'Aquila, Italy.

Preventing hyperoxia-induced regression of retinal vasculature via down regulation of VEGF could preclude the onset of ROP. We tested our hypothesis that dark rearing minimises vaso-obliteration of retinal vessels during hyperoxia as it causes a metabolic sump via depolarising photoreceptors. 10 litters of SD pups were raised in the dark and normal light under hyperoxic conditions (60% and 75% oxygen) from P0-P4 and placed in room air from P4-P8. Blood vessel density and vessel stability were assessed using anti-SMA, NG2, CD39, S100 and GS lectin. Quantitative PCR was used to evaluate levels of VEGF expression. Retina of dark reared rats raised in room air had significantly greater blood vessel density compared to age matched controls (39.4±4.2 vs. 50.8±2.4, p<0.05). When combined with 60% oxygen, vascular density showed no statistical difference compared to pups raised in normal light and room air (39.4±4.2 vs. 38.0±1.2, p>0.05). When dark rearing was combined with 75% oxygen, oxygen flux from the arterial oxygen tension exceeded the increased metabolic demands from photoreceptor depolarisation, resulting in significant vaso-obliteration (33.8±1.6, p<0.05). When neonates returned to room air, dark reared and 60% hyperoxia retina showed near normal mural cell ensheathment whereas at 75% oxygen, abnormal preretinal neovascular formations were seen. Quantitative PCR suggests that dark-rearing modifies levels of VEGF expression. When dark rearing was combined with hyperoxia, physiological growth was mimicked, in terms of vessel density and mural cell ensheathment. Given the non-invasive nature of our treatment, successful application of dark rearing could have an enormous benefit on the visual outcome of premature infants.

## POS-TUE-141

## THE MAMMALIAN EYE AVERAGES COMPETING DEFOCUS

Bowrey H.E.<sup>1</sup>, Tse D.Y.<sup>1,2</sup> and McFadden S.A.<sup>1</sup><sup>1</sup>School of Psychology, The University of Newcastle. <sup>2</sup>School of Optometry, The Hong Kong Polytechnic University.

**AIMS.** Myopia (short-sightedness) can be induced or retarded in animals with spectacle lenses. Minus lenses cause the eye to accelerate its growth and become myopic, while plus lenses have the opposite effect. Using a concentric Fresnel lens with simultaneous positive and negative defocus, we studied whether the mammalian eye can integrate opposite signs of defocus. **METHODS.** 65 guinea pigs raised in a 12/12hr light-dark cycle wore a lens on the right eye from 4-15 days of age. In different groups, the power of the lens either varied in consecutive concentric rings (fresnel dual-power: +5/-5D, 0/-5D, or 0/+5D) or was the same power throughout (single vision: +5D and -5D). Control animals wore only the lens spacer (SP) without any lens. Refractive errors and axial dimensions of the eyes were measured respectively using retinoscopy and ultrasound A-scan after 5 and 11 days of lens-wear. **RESULTS.** Animals wearing -5D lenses became myopic and elongated, while those wearing +5D lenses became hyperopic and relatively shorter. The difference in refractive error between the lens-wearing and fellow-eye for the groups +5D, 0/+5D, +5/-5D, SP, 0/-5D and -5D were 2.73D, 0.48D, -0.72D, -0.09D, -1.58D and -5.68D respectively. Their mean interocular ocular lengths were -0.054 mm, -0.018 mm, 0.041 mm, -0.020 mm, 0.031 mm and 0.073mm respectively. Thus, the dual-power lenses induced less refractive error compensation compared to the corresponding single vision lenses, and competing plus and minus defocus was averaged by the eye. **CONCLUSIONS.** The mammalian eye can integrate opposite optical signals to modulate its growth and refractive error. It implies that optimally designed dual-power lenses will be able to inhibit myopia progression in humans while still providing clearly corrected vision.

## POS-TUE-143

## REGIONAL VARIATION IN SENSITIVITY TO FORM DEPRIVATION MYOPIA IN THE GUINEA PIG

Zeng G.<sup>1,2</sup> and McFadden S.<sup>1</sup><sup>1</sup>School of Psychology, The University of Newcastle. <sup>2</sup>Harbin Medical University.

**Purpose:** Myopia (short-sightedness) can be induced by depriving a growing eye of patterned vision (form deprivation, FD). We have developed a guinea pig mammalian model of FD, and determined whether all parts of the visual field were equally susceptible to FD. **Methods:** Guinea pigs (n=26) were either raised normally or with a diffuser worn over one eye from 7-14 days of age to induce FD. At 14 days of age, refractive error was mapped in the center of the pupil, and off-axis in the superior (S), inferior (I), temporal (T) and nasal (N) visual fields (VF) under cycloplegia. Eye shape was analysed from digital images of frozen sections in both horizontal and vertical planes. **Results:** Untreated animals had more myopia in the I-VF and were hyperopic in the S-VF (I, 0.7±0.5D; S, 6.5±0.3D; N, 2.3±0.3D; T, 3.0±0.3D; C, 2.7±0.4). FD eyes developed central myopia (-7.6±0.7D) and axial elongation (142±29µm) relative to their fellow eye. Peripheral regions varied in their sensitivity to FD, with less myopia and elongation in the N- and T-VF, high sensitivity in the I-VF while the S-VF was resistant to change (N, -3.5±0.7D; T, -3.7±0.6D; S, -0.5±0.7D; I, -6.3±0.9D). This variation was correlated with the difference in the vitreous chamber depth from frozen sections (central; 142±29µm Vs. N, 12±19µm; T, 60±12µm; I, 112±19µm; S, 48±17µm). The sclera perimeter also grew more than twice as much in the retinal area corresponding to the I- than the S-VF (180 Vs 80 µm). **Conclusion:** Peripheral retinal regions vary considerably in their sensitivity to FD. We speculate that the resistance of the superior VF to FD may be related to either the hyperopic set-point and/or the preponderance of blue cones in this retinal region.

## POS-TUE-142

## ONE EXPOSURE PER DAY TO HYPEROPIC BLUR CAUSES MYOPIA

Leotta A.J. and McFadden S.A.

School of Psychology, The University of Newcastle.

**AIMS** Myopia occurs when the eyeball is too long for the power of its optics, so that images are short-focussed. It can be induced if a young growing eye wears a negative spectacle lens which creates hyperopic defocus. The eye compensates by elongating excessively to cancel the defocus. When the lens is removed, the eye is myopic. Using this paradigm, we determined the amount of hyperopic defocus required to induce myopia in the mammalian eye. **METHODS** 136 guinea pigs wore a -4D lens worn on one eye for 12 days. Lenses were worn continuously (n=9), or with an intermittent light cycle designed to vary the exposure (15 min and 1 hr) and signal decay periods (0.25, 1, 2, 4, 6, 23, 35, or 47 hrs of darkness between episodes). Refractive error and ocular dimensions were compared between the two eyes at the end of the lens-wearing period. **RESULTS** Eyes wearing a -4D lens continuously, elongated by 50 µm and developed -4.3D of relative myopia. Significantly more growth (p= 0.02) was induced with one hr exposures every 4 hrs (120 µm growth, -5.1D). Eyes no longer became myopic when the periods between defocus exposures were greater than 42 hrs (18 µm growth, -0.5D). Just 15 minutes or one hr of defocus per day was enough to cause the eye to become myopic (50 µm or 100 µm of growth and -2.9D or -2.7D respectively). **CONCLUSION** The mammalian eye only needs one exposure period per day of hyperopic defocus to induce myopia. This implies that the retinal signal underlying myopia is sustained for long periods, and suggests that if the human eye has brief regular exposures to hyperopic defocus it may induce myopia.

## POS-TUE-144

## FORM DEPRIVATION INDUCES MYOPIA AFTER OPTIC NERVE SECTION IN THE MAMMALIAN EYE

McFadden S.A.<sup>1</sup>, Zeng G.<sup>1</sup>, Ferrandiz N.<sup>1</sup> and Wildsoet C.F.<sup>2</sup><sup>1</sup>School of Psychology, The University of Newcastle. <sup>2</sup>School of Optometry, University of California, Berkeley.

**Purpose:** Myopia develops when a growing eye is deprived of patterned vision (form deprivation, FD). We have developed a mammalian model of myopia using the guinea pig, and ask: Is FD myopia possible when the eye is disconnected from the brain by optic nerve section (ONS)? **Methods:** 15 guinea pigs underwent ONS or Sham surgery at 4-5 days of age. 3 days later, a diffuser was worn on the right eye for 2 weeks. Nine additional animals underwent ONS but did not wear a diffuser. Refractive error and ocular parameters were measured before, during and after FD (at 8, 15 and 22 days of age), and 18 and 33 days after diffuser removal. Retinas were processed for retinal cell degeneration at the completion of the experiment. **Results:** Sham eyes responded like normal eyes to FD (1 week: -4.9D; 2 weeks -5.5D). FD ONS eyes developed excessive myopia (1 week: -7.5D; 2 weeks: -8.5D), some of which could be accounted for by the surgery since ONS alone induced a small amount of myopia (-1.3 to -2.9D). All eyes recovered from their myopia when the diffusers were removed, losing 6.0D (Sham) and 4.9D (ONS) after 18 days with almost complete recovery by 33 days. Severe degeneration of the retinal ganglion cells was found 60 days after ONS. **Conclusions:** Mammalian eyes can respond to FD without the optic nerve, suggesting that acceleration of eye growth from abnormal visual input depends on visual activity intrinsic to the retina. Recovery from FD myopia is also independent of the brain and the retinal mechanisms do not require functional retinal ganglion cells.



## POS-TUE-145

## PERMANENT FUNCTIONAL REORGANIZATION OF RETINAL CIRCUITS INDUCED BY EARLY LONG-TERM VISUAL DEPRIVATION

Di Marco S.<sup>1,2</sup>, Nguyen V.<sup>2</sup>, Bisti S.<sup>1</sup> and Protti D.<sup>2</sup><sup>1</sup>Department of STB, University of L'Aquila, L'Aquila 67100, Italy.<sup>2</sup>Discipline of Physiology and Bosch Institute, University of Sydney, NSW 2006.

**Purpose:** Light deprivation during developmental critical period modifies the response to light stimuli in the retina but is reported to be mostly reversible if visual experience is restored before the end of critical period. To determine if dark-rearing induces permanent rewiring of retinal networks, we characterized its effects on the receptive field (RF) organization and response strength in retinal ganglion cells (RGCs) in controls and in rats born and raised in complete darkness during the critical period and then returned to normal environment (DR/R). **Methods:** Rats were born and raised in complete darkness for 2-4 months and returned to normal circadian rhythm for at least 2-8 months. Rats of matching age were used as controls. Retinas were dissected under infrared light and maintained in oxygenated Ames medium. Light-evoked responses elicited with spots of different diameters were recorded from RGCs in whole-mount retinas either in voltage-clamp or in current-clamp configuration. **Results:** The analysis of area-response function in controls (n=14 cells) and in DR/R animals (n=10 cells) shows a reduction in both the response strength and their RF size ( $p < 0.01$ ). The ratio of inhibition-to-excitation for control (n=8 cells) and DR/R animals (n=13 cells) was significantly different ( $p < 0.05$ ). The spatial distribution of excitatory inputs in controls is similar to the one of DR/R animals, while inhibitory inputs from DR/R are spatially disorganised compared to control animals. **Conclusions:** These results show that early visual experience is critical for the refinement of retinal circuits, and suggest that abnormal visual experience during the critical period impacts on retinal network and consequently on vision.

## POS-TUE-146

## FEATURES OF THE HUMAN ROD BIPOLAR CELL ERG RESPONSE DURING FUSION OF SCOTOPIC FLICKER

Cameron A.<sup>1,2</sup>, Lam J.<sup>1,3</sup> and Campion M.<sup>1,3</sup><sup>1</sup>The ARC Centre of Excellence in Vision Science. <sup>2</sup>Discipline of Physiology, Bosch Institute, The University of Sydney. <sup>3</sup>The John Curtin School of Medical Research.

The ability of the eye to distinguish between intermittently presented flash stimuli is a measure of the temporal sensitivity of vision. The aim of this study was to examine the relationship between summation of the human rod bipolar cell response (as measured from the scotopic ERG *b*-wave) and the psychophysically measured critical fusion frequency (CFF). Stimuli consisted of dim ( $\sim 0.35$  Rh\* per rod), blue flashes presented either singly, or as flash pairs (at a number time separations, between 5 - 200 ms). Single flashes of double intensity ( $\sim 0.70$  Rh\* per rod) were also presented as a reference. Visual responses to flash pairs were measured via (1) recording of the ERG *b*-wave, and (2) threshold determinations of the CFF using a two-alternative forced-choice method (flicker vs. steady illumination). Participants were healthy adults, with normal or corrected-to-normal visual acuity, from whom informed written consent was obtained. The results of this experiment suggest that ERG *b*-wave responses to flash pairs separated by  $< 100$  ms undergo response summation, consistent with the threshold for the CFF; while those of shorter duration ( $< 50$  ms) may be electrophysiologically similar to presenting a single flash of double the intensity. In conclusion, the visual system's ability to discriminate between scotopic stimuli may be determined by the response characteristics of the rod bipolar cell, or before, by the rod photoreceptor itself.

## POS-TUE-147

## SPATIAL AND TEMPORAL STIMULUS VARIANTS OF MULTIFOVAL PUPILLOGRAPHIC PERIMETRY

Sabeti F.<sup>1,2</sup>, Maddess T.<sup>1,2</sup>, Essex R.<sup>3,4</sup> and James A.<sup>1,2</sup><sup>1</sup>Centre for Visual Sciences, ANU, Canberra, Australia. <sup>2</sup>ARC Centre of Excellence in Vision Science, Canberra, Australia. <sup>3</sup>College of Medicine, Biology and Environment, ANU, Canberra, Australia.<sup>4</sup>Department of Ophthalmology, The Canberra Hospital, Canberra, Australia.

**Purpose:** To investigate pupillary responses of dichoptic multifocal pupillometry to spatial and temporal stimulus variants in normal subjects. **Methods:** Peak pupillary constriction amplitudes, time to peak and width of contractions were analysed for 29 normal (mean age 70.9  $\pm 6.0$ ) subjects with 4 different stimulus protocols. Stimuli were presented dichoptically and pupil responses were measured concurrently. All protocols presented multifocal stimuli with a dartboard layout having 24 or 44 independent test regions/eye with a mean presentation interval of 1 or 4 s/region and a presentation duration of 33 ms, subtending  $\pm 15^\circ$  of visual field. Luminance of the stimulus regions was 250 cd/m<sup>2</sup> and background 10 cd/m<sup>2</sup>. **Results:** Stimuli presented in a 24 region layout with a 4 s/region presentation rate achieved the largest mean amplitude, shortest time to peak and response width. These measures were compared with a reference stimulus which consisted of a 44 region array at 1 s/region presentation rate. Relative to this reference, the 24 region 4 s/region presentation rate increased amplitudes by 3.5x ( $b = 5.56$  dB); and decreased latencies by 76.0x ( $b = 18.81$  dB); and width of responses decreased by 24.5x ( $b = 13.89$  dB), all with  $p < 0.00001$ . Median signal to noise ratios expressed as Z-scores of a normal distribution per protocol ranged from 1.73 to 3.15 with 4 s/region presentation rates achieving the highest Z-scores. **Conclusion:** Long duration stimulus presentation rates with low resolution layouts produce the largest effect on amplitudes, time to peak, and response widths.

## POS-TUE-148

## PUPILLARY RESPONSES TO BLUE STIMULATION USING MULTIFOVAL PUPILLOGRAPHIC OBJECTIVE PERIMETRY

Carle C.F.<sup>1,2</sup>, James A.C.<sup>1,2</sup> and Maddess T.L.<sup>1,2</sup><sup>1</sup>Eccles Institute of Neuroscience, ANU, Canberra, Australia. <sup>2</sup>ARC Centre of Excellence in Vision Science, Canberra, Australia.

**Purpose:** To assess the effect of stimulus luminance and duration on pupillary responses of normal subjects using blue multifocal pupillometric objective perimetry (mfPOP) stimuli. Slow blue stimuli are of interest given the possible contribution of melanopsin containing retinal ganglion cells to mfPOP responses. **Methods:** Five normal subjects were tested in two experiments (n=1, n=5) comprising 13 stimulus protocols and two different stimulus layouts (24 regions, 44 regions). The characteristics of pupillary response waveforms to various combinations of stimulus durations (750-2667ms) and luminances (40-80cd/m<sup>2</sup>) were investigated. Effects were quantified using a multivariate linear model. **Results:** Longer stimulus durations resulted in significantly longer times to peak of the contraction (n=1: 1.34ms delay per 100ms increased duration,  $t(838) = 5.73$ ,  $p < .0001$ ). Longer stimulus durations also resulted in small but significant reductions in pupil contraction amplitudes (n=1: -0.24 $\mu$ m per 100ms increased duration,  $t(838) = -2.68$ ,  $p < .01$ ). Increased luminance produced significantly larger amplitudes (n=1: 2.55 $\mu$ m per 10cd/m<sup>2</sup>,  $t(838) = 5.73$ ,  $p < .0001$ ; n=5: 4.74 $\mu$ m per 10cd/m<sup>2</sup>,  $t(3475) = 9.45$ ,  $p < .0001$ ) as well as significantly shorter times to peak (n=5: -8.3ms per 10cd/m<sup>2</sup>,  $t(3475) = -14.79$ ,  $p < .0001$ ). Response waveforms at longer pulse durations displayed a sustained component which ended with a small contraction. The period between response onset and the offset contraction corresponded to the stimulus duration in each case, indicating that the integration time of the pupillary system had been exceeded. Contraction amplitudes were much smaller or not detectable to stimulation of the parafoveal retina. **Conclusions:** Pupillary responses to blue multifocal stimuli vary with changes in stimulus luminance and duration. The largest, fastest contractions were obtained to brighter, shorter stimuli.

## POS-TUE-149

**ALTERED IPSILATERAL TOPOGRAPHY, INDUCED BY THE DELETION OF TEN-M3, LEADS TO THE EMERGENCE OF NOVEL OCULAR DOMINANCE DOMAINS IN MICE**

Merlin S.<sup>1</sup>, Horng S.<sup>2</sup>, Marotte L.R.<sup>3</sup>, Sur M.<sup>2</sup>, Sawatari A.<sup>1</sup> and Leamey C.A.<sup>1</sup>

<sup>1</sup>Bosch Institute & Discipline of Physiology, University of Sydney, Sydney. <sup>2</sup>Brain & Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA, USA. <sup>3</sup>Research School of Biology, Australian National University, Canberra.

The visual system is characterised by a precise, topographically appropriate representation of the visual field. We recently showed that deletion of Ten-m3 causes mismapping of the ipsilateral terminals in the thalamus, and is associated with deficits in visual behaviour. Notably, monocular-inactivation rescues these deficits. Transneuronal tracing showed that ipsilateral inputs map aberrantly to the medial, typically monocular, region of primary visual cortex (V1) in Ten-m3 knockout (KO) mice. Terminals were grouped in clusters that spanned V1 (n=4), different to WT (n=4) where a single patch of terminals was always seen in lateral V1 (p<0.01; Multivariate ANOVA). Immunohistochemistry for c-fos in monocularly-inactivated mice revealed discrete clusters of ipsilaterally-driven cells in medial V1 of KOs (n=6) but not in WTs (n=6, p<0.01, Multivariate ANOVA). Clusters of low reactivity were seen contralaterally in KOs. In vivo single-unit recordings revealed that single V1 neurons receive disparate inputs via each eye in KOs; the mean separation of receptive fields ( $25.9^\circ \pm 3.7^\circ$ , median  $18.0^\circ$ ; 32 cells) was significantly increased compared to WT ( $9.5^\circ \pm 2.2^\circ$ , median  $0^\circ$ ; 25 cells; p<0.01; Mann-Whitney U-test). A significant shift in the monocularly index (p<0.01, Kolmogorov-Smirnov test) was also observed. Intrinsic optical imaging revealed complementary regions of strongly ipsilateral or contralateral drive in V1, consistent with an increase in monocularly. We propose that the subcortical ipsilateral mismapping drives the emergence of an ocular dominance structure in Ten-m3 KOs.

## POS-TUE-151

**A MODEL FOR SIGNAL PROCESSING IN PRIMARY VISUAL CORTEX**

Hesam Shariati N. and Freeman A.W.  
University of Sydney.

**Aim.** Existing models of primary visual cortex describe orientation selectivity, direction selectivity, and complex responses, in individual cortical cells. The models tell us less, however, about population properties. Our aim is to produce a mathematical model for primary visual cortex that not only yields cortical cells with these fundamental properties, but also explains the diversity of behaviour across the population. **Methods.** The model consists of four sub-cortical stages - photoreceptors, bipolar cells, ganglion cells, and lateral geniculate nucleus - and three cortical stages. There are two sub-cortical channels, one relaying off-centre signals and the other on-centre signals. Each cortical stage comprises a rectangular grid of neurons with spacing substantially smaller than the distance between the two geniculate inputs. The input to each stage is convergent, with a Gaussian spread of synaptic weights. Each neuron in the model is a low-pass temporal filter implemented by one differential equation; the output of cortical neurons is rectified. **Results.** The model simulates the direction selectivity by assuming that signal processing in one sub-cortical channel is slower than in the other. This also results in a range of selectivities across cells. The resulting cortical cells have elongated on- and off-subfields resulting in orientation selectivity. The orientation tuning curve also confirms this property. Responses in cortical stage 1 are simple-like, in that they are strongly modulated by a drifting grating, and the responses become progressively more complex-like in stages 2 and 3 because of signal rectification at each stage. **Conclusion.** This model, representing one cortical column, simulates three fundamental properties of primary visual cortex. It also shows the diversity of direction selectivity and the origin of complex-like behaviour.

## POS-TUE-150

**ANATOMICAL AND PHYSIOLOGICAL CHANGES IN THE VISUAL SYSTEM FOLLOWING DAMAGE TO THE PRIMARY VISUAL CORTEX (V1) EARLY IN LIFE**

Foo D.C.<sup>1,2</sup>, Homman-Ludiye J.<sup>2</sup> and Bourne J.A.<sup>2</sup>

<sup>1</sup>Department of Anatomy & Developmental Biology, Monash University, Clayton 3800, Australia. <sup>2</sup>Australian Regenerative Medicine Institute, Monash University, Clayton, Vic, 3800, Australia.

Injury to the primary visual area (V1) is common following hypoxia, traumatic brain injury and stroke in humans. Although some sparing of visual functions occurs in adults, a more robust recovery occurs in neonatal humans and nonhuman primates, which suggests that the brain loses its plastic capabilities with age. To assess this, we identified anatomical, cellular and functional alterations in extrastriate areas of the visual cortex following neonatal (PD10, n=2) and adult (4 yrs, n=2) V1 unilateral lesions in the marmoset monkey (*Callithrix jacchus*). Using immunohistochemistry, we were able to identify changes in a subset of excitatory neurones by their expression of nonphosphorylated neurofilament, and two subsets of inhibitory interneurons that express either calbindin or parvalbumin. Functional visual activity was determined by detecting the expression of the Fos protein, potentially activated by neural stimulation. Following V1 lesion, changes in both excitatory and inhibitory neurones in the contralateral V1, second visual area (V2) and middle temporal area (MT) were detected, suggesting a role for transcallosal projections. The ipsilateral hemisphere of the neonatal animal closely resembles the control, demonstrating a high capacity for plasticity. More importantly, we detected alterations in the adult lesioned animal, revealing that the adult still retains some plastic capabilities. Using the Fos protein, we have been the first to show changes in the activity of excitatory and inhibitory interneurons following a V1 lesion in MT of nonhuman primates. These findings highlight the role of both excitatory and inhibitory neurones in plasticity following a cortical injury and suggest that alterations in their regulation have the potential to re-induce developmental plasticity in the adult brain.

## POS-TUE-152

**MULTIPLE LINEAR REGRESSION FOR ANALYSING CORTICAL RECEPTIVE FIELDS: BETTER THAN YOUR AVERAGE SPIKE-TRIGGERED AVERAGE**

Van Kleef J.P., Cloherty S.L., James A.C. and Ibbotson M.R.  
ARC Centre of Excellence in Vision Science, Research School of Biology, Australian National University Canberra, ACT 2601, Australia.

Simple and complex cells are the two dominant classes of neuron found in the primary visual cortices of mammals. These cell types can be distinguished by the way they spatially and temporally integrate contrast stimuli that are both brighter (ON stimuli) and darker (OFF stimuli) than the mean background - their receptive field (RF) properties. For example, simple cells have spatially distinct ON and OFF zones whereas complex cells have ON and OFF zones that mostly overlap. Simple cells have linear RF properties that are analytically easier to evaluate than the nonlinear RF properties of complex cells. Here we demonstrate a novel white noise technique that enables previous linear analyses to be extended to evaluate the ON and OFF RFs of complex cells. We recorded extracellular responses from complex cells (n=8) in cat visual cortices (areas 17 and 18) to pseudorandom stimuli and estimated their RFs using the extended spike-triggered average (STA) and multiple linear regression (MLR) methods. We found that in all cells, the correlation coefficient between the measured response and the response predicted using the extended MLR method was higher than it was for the extended STA method. Furthermore, the correlation coefficients for the extended MLR method are comparable to more complicated techniques such as spike-triggered covariance (STC). We suggest that given the ON and OFF segregation in the visual pathways of mammals, the two linear RF subunits (ON and OFF) produced by our novel method are more biologically plausible than the multiple linear subunits produced using the STC method.

## POS-TUE-153

**RESOLUTION OF V1 AND V2 ACTIVITY IN HUMAN VISUAL CORTEX BY INTEGRATION OF MULTIFOCAL FMRI, EEG AND MEG NEUROIMAGING**James A.C.<sup>1</sup>, Goh X.-L.<sup>1</sup>, Henriksson L.<sup>2</sup> and Vanni S.<sup>2</sup><sup>1</sup>ARC Centre of Excellence in Vision Science, and Research School of Biology, The Australian National University. <sup>2</sup>Brain Research Unit, Low Temperature Laboratory and Advanced Medical Imaging Centre, Helsinki University of Technology, Helsinki, Finland.

Distinguishing the contribution of cortical areas in evoked response studies in human neuroimaging is made difficult due to the close proximity of the areas involved, the complex folding of the cortical sheet, and the largely overlapping time-course of responses. We used multifocal methods we have now developed [1, 2] to image activity in visual cortex of six subjects separately in three modalities, each with an identical spatial layout stimulating 60 regions of the visual field. Regions were concurrently stimulated, in a block design for fMRI [2] and in a pattern-pulse design for EEG and MEG recording, similar to [1]. Coregistration of fMRI activation volumes [2] with high resolution anatomical scans gave the mapping of areas V1 and V2 on the cortical sheet for each subject. EEG responses on 74 channels (BioSemi) and MEG on 306 channels (Neuromag) were decomposed to estimate elementary responses for each region using multiple linear regression [1]. Source currents within V1 and V2 were estimated using a novel equivalent normal vector method integrating over curved patches of cortical sheet, to give waveforms of current dipole density per unit area. V1 downward current peaked at 80-95ms, while V2 current had a prominent upward peak earlier, at 70-80ms. Peak dipole current density estimated from the independent methods of EEG and MEG corresponded remarkably closely, at 0.2-0.3 nAm/sqmm. 1. James AC, 2003. The pattern-pulse multifocal visual evoked potential. *IOVS*, 44(2), 879-890. 2. Vanni S, Henriksson L and James AC, 2005. Multifocal fMRI mapping of visual cortical areas. *Neuroimage*. 27(1):95-105.

## POS-TUE-154

**THE FOVEAL CONFLUENCE IN PRIMATE, INVESTIGATING, MODELING, EXPLAINING**Schira M.M.<sup>1,2</sup>, Tyler C.W.<sup>3</sup>, Spehar B.<sup>2</sup> and Breakspear M.<sup>1,4</sup><sup>1</sup>School of Psychiatry and Black Dog, University of New South Wales. <sup>2</sup>School of Psychology, University of New South Wales. <sup>3</sup>The Smith Kettlewell Eye Research Institute, San Francisco. <sup>4</sup>Queensland Institute of Medical Research and the Royal Brisbane and Women's Hospital, Queensland.

**Background:** A basic organizational principle of the primate visual system is that it maps the visual environment repeatedly and retinotopically onto cortex. Simple algebraic models can be used to describe the projection from visual space to cortical space not only for V1, but also for the complex of areas V1, V2 and V3. Typically a conformal (angle-preserving) projection ensuring local isotropy is regarded as ideal and primate visual cortex is often regarded as an approximation of this ideal. Using high resolution fMRI (1.2x1.2x1.2mm) we demonstrated systematic deviations from this ideal that are especially relevant in the foveal projection (Schira et al. *J. Neurosci.* 2009). Here we present and investigate a simple algebraic model that accurately predicts the observed data. **Methods/Findings:** The retino-cortical map can be optimized towards a space-conserving homogenous representation or a quasi-conformal mapping. The latter would require a significantly enlarged representation of specific parts of the cortical maps, which is not supported by empirical data. Further, the recently published principal layout of the foveal singularity cannot be explained by existing models. We suggest a new model that accurately describes foveal data, minimizing cortical surface area in the periphery but suggesting that local isotropy dominates the most foveal part of the projection at the expense of additional cortical surface. **Significance:** The foveal confluence is an important example of the detailed trade-offs between the compromises required for the mapping of environmental space to a complex of neighboring cortical areas. Our models demonstrate that the organization follows clear principles that are essential for our understanding of foveal vision in daily life.

## POS-TUE-155

**DEVELOPMENT OF THE SPECIALISATION OF CENTRAL PRIMATE RETINA**Kozulin P.<sup>1</sup>, Natoli R.<sup>1</sup>, Madigan M.C.<sup>2</sup>, Bumsted O'Brien K.M.<sup>1</sup> and Provis J.M.<sup>1</sup><sup>1</sup>Research School of Biology and ARC Centre of Excellence in Vision Science, ANU, Canberra ACT. <sup>2</sup>School of Optometry and Vision Science, UNSW, Kensington NSW.

**Purpose:** Three overlapping phases of development characterize the morphological specialization of the macula: (1) ganglion cell (GC) axon pathfinding in the retina; (2) definition of the foveal avascular area, and (3) retinotopic mapping onto visual targets. We aimed to identify candidate genes with roles in these different phases. **Methods:** We carried out a microarray analysis, using human fetal RNA at 19-20 weeks' gestation (n=4), to identify genes differentially expressed in the macula and confirmed expression by quantitative RT-PCR (QPCR) and by *in situ* hybridisation, using macaque retinas aged between fetal day 55 and adulthood. **Results:** Gradients of mRNA expression in the GC layer were observed for the axon guidance genes EphA6, unc5h4 and netrin G1, which changed over time. EphA6 was highly expressed in the macula during fetal life and levels of expression in the macula increased postnatally. Netrin G1 was highly expressed early in fetal life, but decreased postnatally. Unc5h4 was highly expressed in the macula during formation of the avascular area, but was low in early development and postnatally. The anti-angiogenic factors pigment epithelium-derived factor (PEDF) and brain natriuretic protein (BNP) were highly expressed in the macula during development and postnatally. **Conclusion:** Changing levels of expression of these genes in the macula during pre- and postnatal life suggests they have sequential roles in the three phases of development. The data suggest that EphA6 regulates vascular patterning early in development and characterises the projection from foveal GC in the postnatal phase. The findings give insight into how the characteristics of the macula may have evolved.

## POS-TUE-156

**FACILITATION IN HYPERACUITY OF DRAGONFLY HYPERCOMPLEX NEURONS**Dunbier J.R.<sup>1</sup>, Bolzon D.M.<sup>1</sup>, Wiederman S.D.<sup>1</sup>, Nordstrom K.<sup>1,2</sup> and O'Carroll D.C.<sup>1</sup><sup>1</sup>Discipline of Physiology, The University of Adelaide, SA, 5005 Australia. <sup>2</sup>Department of Neuroscience, Uppsala University Biomedical Centre, Box 593, 75124 Uppsala, Sweden.

We recently identified similarities in processing of target motion by STMD (small target motion detector) neurons in the insect lobula complex (3rd optic ganglion) and hypercomplex cells in the mammalian cortex [1]. One surprising feature is that such neurons achieve selectivity for small features at the limits for eye resolution. We recently argued that this is achieved through powerful mechanisms of local inhibition [2,3]. However, 'target hyperacuity' (the response to 'sub-pixel' features) requires neural mechanisms to amplify very low contrasts in the image (<1%), i.e. extremely high contrast gain. How this is achieved whilst maintaining lack of spontaneous activity remains poorly studied, but our modelling suggests that such a mechanism is a pre-requisite for reliable detection once receptor noise is accounted for. One possibility is that gain is facilitated by higher order interactions between local motion detecting elements. To test this, we used recordings from dragonfly STMD neurons and single target stimuli that either drifted through the receptive field, or which began at discrete locations within it. By normalizing responses for the receptive field shape (determined with the drifting stimulus) we show that responses are facilitated by very slow mechanisms. Best fits to data for one identified neuron (CSTMD1) suggest time constants for this facilitation on the order of 300 ms ( $T_{50} = 217\text{ms}$ ,  $N=4$ ) - several times slower than the neural delay intrinsic to local motion detection. [1] Nordström & O'Carroll (2009) *Trends in Neurosciences* 32: 383-391 [2] Wiederman et al. (2008) *PLoS ONE* 3, 7, e2784 [3] Bolzon et al. (2009) *Journal of Neuroscience* (in press).



## POS-TUE-157

## RECLASSIFICATION OF SIMPLE AND COMPLEX CELLS IN THE PRIMARY VISUAL CORTEX OF THE CAT

Hietanen M.A.<sup>1,2</sup>, Van Kleef J.<sup>1,2</sup> and Ibbotson M.R.<sup>1,2</sup><sup>1</sup>Visual Sciences, Research School of Biology, Australian National University, Canberra, ACT, 2601, Australia. <sup>2</sup>ARC Centre of Excellence in Vision Science.

Ever since Hubel and Wiesel noted that neurons in the primary visual cortex fit into two distinct cell types (simple and complex) it has been standard for authors to divide their cell populations into those two groupings. Neurons are most often divided into simple and complex cells based on their responses to drifting gratings. Current quantitative techniques decompose the response into its mean (F0) and the first Fourier component (F1), which oscillates at the temporal frequency of the grating. The cell is then classified as simple if  $F1/F0 > 1$  or complex if  $F1/F0 < 1$ . We show that in an ideal model of a complex cell both the mean and variance of the  $F1/F0$  ratio increase as the spike count decreases. We show analytically that the expected  $F1/F0$  ratio for a cell that spikes  $n$  times in a grating cycle is  $2/\sqrt{n}$ . A new quantitative classification technique is presented in which cells are classified as simple based on their relationship between spiking and  $F1/F0$ . We test this new definition with a large sample ( $n=468$ ) of neurons in the primary visual cortex of the cat, demonstrating that there is a clear relationship between cell classification and the neuron's laminar location that is more clearly revealed using this new classification scheme. Specifically, simple cells are predominantly found within deeper laminar when examined using the new definition, and relatively evenly distributed across layers when the spiking of the cell is not used in the classification of the cell. As our technique only requires extracellular recording and uses moving sine wave gratings, to which cortical cells respond very strongly, we believe that it should be considered for adoption as the standard classification technique in cortex.

## POS-TUE-159

## STRUCTURE OF EXTRA-CLASSICAL RECEPTIVE FIELDS OF NEURONES IN CAT'S AREA 18

Zeater N.<sup>1,2</sup>, Romo P.A.<sup>1,2</sup>, Solomon S.G.<sup>1,2</sup>, Wang C.<sup>1,2</sup> and Dreher B.<sup>1,2</sup><sup>1</sup>School of Medical Sciences & Bosch Institute, University of Sydney, NSW, 2006, Australia. <sup>2</sup>ARC Centre of Excellence in Vision Science, University of Sydney, NSW, 2006, Australia.

The 'hypercomplex' cells of the mammalian visual cortex were originally described as complex cells with silent, suppressive regions on one or both ends of the discharge (spike-generating) field, along the axis of a cell's optimal orientation<sup>1</sup>. **Purpose:** To examine the spatial structure of silent, extra-classical receptive fields (ECRFs) and its relation to the 'hypercomplexity' of single neurones in the parastriate part (area 18, V2) of cat primary visual cortex. **Methods:** Single neurones, recorded from area 18 of anaesthetized and immobilized adult cats, were identified on the basis of the ratio of the phase-variant (F1) component to the mean firing rate (F0) of their spike-responses to patches of optimised, achromatic sine-wave gratings drifting through their receptive fields (simple:  $F1/F0 > 1$ ; complex  $F1/F0 < 1$ ). **Results:** The majority of cells tested (26/34; 18 of them, simple<sup>2</sup>) could be identified as 'end-stopped' since presentation of patches of gratings restricted to the silent subregion(s) along the axis of the optimal orientation, reduced substantially (>30%) the magnitude of responses to the gratings restricted to the spike-generating regions. However, in the great majority of these (22/26), suppressive subregions were not confined to the regions along the axis of optimal orientation (cf. 'higher-order' hypercomplex cells<sup>1</sup>). **Conclusions:** The higher-order hypercomplexity of area V2 cells does not necessarily imply the higher-order status of the area<sup>1</sup>. Indeed, the ECRFs of most area 18 neurones, like the ECRFs of their afferent neurones in the dorsal lateral geniculate nucleus, appear to completely surround the discharge regions. <sup>1</sup>Hubel DH and Wiesel TN (1965) J. Neurophysiol., 28, 229-289. <sup>2</sup>Dreher B (1972) Invest. Ophthalmol., 11, 355-356.

## POS-TUE-158

## CONTRAST RESPONSE FUNCTIONS FOR GRATINGS AND PLAIDS IN HUMAN VISUAL CORTEX

McDonald J.S.<sup>1</sup>, Mannion D.J.<sup>1,2</sup> and Clifford C.W.G.<sup>1,2</sup><sup>1</sup>School of Psychology, University of Sydney, Sydney NSW 2006, Australia. <sup>2</sup>Australian Research Council Centre of Excellence in Vision Science.

How do visual systems code the contrast of different patterns? A recent intrinsic signal optical imaging study in tree shrew showed surprisingly that the population response of V1 to plaid patterns comprising orthogonal grating components of equal contrast is predicted by the average of the responses to the individual components (MacEvoy *et al.*, 2009). This prompted us to compare responses to plaids and gratings in human visual cortex as a function of contrast. We used fMRI at 3T to measure the BOLD response of retinotopically-defined regions in 8 subjects. We found that the responses of areas V1-V3 to a plaid comprising superposed orthogonal grating components of equal contrast were on average 15-30% higher than the responses to a single grating of the same contrast as the components. However, the response to a plaid was predicted to within 4% by the response to a grating of twice the contrast of the plaid components. These data show that in humans the fMRI BOLD response of early visual cortex to plaid patterns is not the average of the response to the components. Instead, the population response to a given pattern appears to depend on the contrast energy within that pattern regardless of whether that energy is distributed over one or more orientations. Reference: MacEvoy SP, Tucker TR, Fitzpatrick D. (2009) A precise form of divisive suppression supports population coding in the primary visual cortex. *Nat Neurosci.* 12:637-645.

## POS-TUE-160

## DISTINCT MECHANISMS UNDERLYING THE EFFECT OF VISUAL MOTION ON PERCEIVED POSITION

Huby A., Holcombe A.O. and Linares D.  
School of Psychology, University of Sydney.

As the visual system suffers from substantial neural latencies (~100 ms), by the time a moving object is processed, its position will have changed significantly. To explain various illusions, a popular theory is that the brain shifts perceived position of moving objects in the direction of its motion to overcome neural latencies. For latency compensation, shifts should increase with speed. The flash-lag illusion – where a flash is perceived to lag a moving object despite being aligned – does increase with speed. However, this is also consistent with an attentional shift unrelated to compensation. We investigated the speed dependence of a related illusion (flash-drag), where nearby irrelevant motion shifts the perceived position of flashed objects. The flash-drag cannot be explained by an attentional shift, so its speed dependence is a critical test of compensation theories. **EXPERIMENTS.** Dots above fixation moved to the right while dots below fixation moved to the left. Two flashed bars were presented near each dot field. Consistent with the flash-drag, participants ( $n=5$ ) perceived the flashes biased in the direction of the nearby motion. Unlike the flash-lag illusion, which increases rapidly over a wide speed range, the flash-drag saturated at a slow 5°/sec. Furthermore, by using a fast speed (9°/sec) and alternating the motion direction of the dots between right and left at slower and slower rates, we found that the flash-drag did not saturate until 1.5 Hz, which suggests that a longer interval of motion (300ms) is used than in the flash-lag effect (80 ms). **CONCLUSIONS.** As the flash-drag saturates at slow speeds and integrates motion for an extended interval after the flashes, it might not reflect compensation for neural latencies.

## POS-TUE-161

**DOES THE PULVINAR NUCLEUS CONTRIBUTE TO THE EARLY MATURATION OF THE DORSAL STREAM VISUAL CORTICAL AREAS?**Warner C.E.<sup>1,2</sup> and Bourne J.A.<sup>2</sup><sup>1</sup>Department of Anatomy and Developmental Biology, Level 3 Building 76 (STRIP 2), Monash University, Victoria, 3800, Australia. <sup>2</sup>Australian Regenerative Medicine Institute, Level 1 Building 75 (STRIP 1), Monash University, Victoria, 3800, Australia.

In the present study, we demonstrate the perinatal development of the retinopulvinar pathway to the middle temporal visual cortical area (MT) and morphological development of the pulvinar nucleus. Marmoset monkeys (*Callithrix jacchus*) aged between embryonic day 130 and 9 months (n=9) were injected with fluorescently conjugated CTb and fast blue in the eyes and left hemisphere area MT, respectively. Following a one week survival period, animals were perfused, their brains frozen and coronal sections processed immunohistochemically and histologically to assist in the demarcation of thalamic nuclei boundaries. Colocalisation of fluorescently labelled contralateral retinal ganglion terminals and area MT relay cells was confirmed using synaptophysin and statistical analyses of resultant confocal images. In the medial nucleus of the pulvinar (Plm) we observed dense labelling of relay cells to ipsilateral MT area. In the adult a small number of these cells were recipient of retinal projections predominately from the contralateral eye whereas younger animals received increased labelling of binocular input to the Plm as well as to neighbouring subnuclei of the pulvinar nucleus. Furthermore, it was confirmed that contralateral retinal afferents formed synaptic connections with labelled area MT relay cells in the Plm. These results provide evidence of the early development of a direct extrageniculate pathway from the retina to area MT early in life, prior to the maturation of all visual cortical areas. Furthermore, these data demonstrate an alternate pathway that may be responsible for the early maturation of the dorsal stream cortical areas.

## POS-TUE-162

**THE RESPONSES OF MT COMPONENT AND PATTERN CELLS TO TRANSPARENTLY MOVING DOTS**McDonald J.S.<sup>1</sup>, Clifford C.W.G.<sup>1,2</sup>, Camp A.J.<sup>3</sup>, Tailby C.<sup>4</sup>, Coorey N.J.<sup>3</sup> and Solomon S.G.<sup>2,3</sup><sup>1</sup>School of Psychology, University of Sydney, Sydney NSW 2006, Australia. <sup>2</sup>Australian Research Council Centre of Excellence in Vision Science. <sup>3</sup>Discipline of Physiology, School of Medical Sciences and Bosch Institute, The University of Sydney, Sydney NSW 2006, Australia. <sup>4</sup>National Vision Research Institute, Carlton, VIC 3053, Australia.

MT Neurons can be categorized into two types on the basis of their response to drifting plaid patterns; those which respond to the direction of the individual grating components (component cells) and those which respond to the average motion (pattern cells). Here, we investigated how these categories of neurons respond to moving transparent dot-fields. Standard preparation techniques were used (Camp et al, J. Neurosci. 2009, 29(15):5009-5021) for electrophysiological recording from MT in 4 marmosets. Neuronal signals were isolated and their optimal spatio-temporal parameters were quantified. On the basis of their direction tuning to single gratings and to plaids, neurons were categorized into component and pattern cells. Finally the responses of the neurons to 2 transparent fields of moving dots (120 degrees apart), at a range of speeds were measured. The component cells tended to respond optimally to the average direction of the two planes of dot-fields and pattern cells generally responded optimally to the individual dot-fields. Remarkably, this pattern of responding is the reverse of the grating/plaid case. If, however, the speed of the stimulus was far from the optimal speed of the pattern cell, then its direction tuning often resembled that of a component cell. These responses, both to grating/plaid and transparent dot-fields, can be accounted for by the model of Simoncelli and Heeger (Vis. Res. 1998, 38(5):743-761), both at optimal and non-optimal speeds.

## POS-TUE-163

**PARALLEL SUB-PATHWAYS OF S-CONE SIGNALS TO THE MACAQUE'S MIDDLE TEMPORAL AREA**Jayakumar J.<sup>1</sup>, Roy S.<sup>1</sup>, Dreher B.<sup>2</sup> and Vidyasagar T.R.<sup>1</sup><sup>1</sup>Department of Optometry & Vision Sciences, University of Melbourne, Vic 3010. <sup>2</sup>School of Medical Sciences & ARC CoE in Vision Science, University of Sydney, NSW 2006.

We have recently demonstrated (Roy et al., Eur. J. Neurosci., 30, 1517-1526, 2009) that in the macaque, the short-wavelength sensitive cone (S-cone) signals from the retina are sent largely to the koniocellular regions of the dorsal lateral geniculate nucleus (LGN). In the present study, we investigated whether the S-cone signals to the cortical middle temporal area (MT) are relayed via the primary visual cortex (area V1) or reach MT bypassing V1, such as through the direct projections to MT from the koniocellular layers of the LGN (Sincich et al., Nat. Neurosci., 7, 1123-1128, 2004). Twenty-one cells in our sample of MT cells recorded in the anaesthetized and immobilized macaques showed significant responses to S-cone isolating stimuli. We studied their responses before and during reversible inactivation (by cooling) of V1. Twelve of these S-cone input cells showed a significant reduction in the magnitude of response to the S-cone isolating stimuli during cooling. The rest of the cells showed no significant change in response to S-cone stimuli during cooling. The former group, which presumably receive their S-cone signals via area V1 had longer response latencies ( $81.5 \pm 38.3$ ms) compared to the latter group whose S-cone signals bypass V1 ( $57.8 \pm 29.5$ ms). This difference in latencies, however, failed to reach statistical significance (Student t test;  $p=0.12$ ). Our results indicate that the S-cone signals might reach MT via at least two distinct pathways, one through V1 and the other bypassing V1. The alternative route(s) may be via the LGN and/or the pulvinar.

## POS-TUE-164

**HOW MOTION INTEGRATION BY NEURONS IN PRIMATE AREA MT DEPENDS ON TEMPORAL FREQUENCY**Solomon S.G.<sup>1</sup>, Camp A.J.<sup>1</sup> and Tailby C.<sup>2</sup><sup>1</sup>Bosch Institute, The University of Sydney. <sup>2</sup>NVRI, The University of Melbourne.

Some cells in the middle temporal area (MT) of macaque are selective for the overall motion of a visual pattern, while others respond to the motion of the components of that pattern. Yet while MT cells have broad temporal frequency (TF) tuning, it is not known how the motion integration depends on TF. We addressed this by making extracellular recordings from 100 cells in the middle-temporal region of four opiate-anaesthetized adult male marmosets (*Callithrix jacchus*); direction tuning curves were measured for brief presentations of 50% contrast sinusoidal gratings drifting at each of four TF (3, 6, 12, & 25 Hz), and plaids made of two gratings that drifted at the same TF in directions 120 degrees apart. From these responses we calculated a pattern-selectivity index, and determined how this index evolved over the first 300 ms of response [Smith MA, Majaj NJ, Movshon JA (2005) Dynamics of motion signaling by neurons in macaque area MT. Nat Neurosci, 8:220-8]. We first established that the pattern/component distinction holds in the marmoset, a diurnal New World primate: of cells that could be classified, 37 were component-selective, 17 were pattern-selective, and 5 responded to plaids but not the component gratings. Among individual cells motion integration was generally stable across the TF to which the cell responded – for those that responded robustly to both, the index at 3 Hz predicted that obtained at 25 Hz ( $r = 0.84$ ,  $n = 33$ ) – but motion integration was always more pronounced at low TF. The response of component cells was transient, and component cells could be reliably classified from the very earliest part of their response. The response of pattern cells was more sustained, and it took longer for their response to clearly signal motion integration; this lag was most pronounced at low temporal frequencies.

S

## POS-TUE-165

**ADAPTATION TO SURFACE MOTION PERCEIVED THROUGH TOUCH**McIntyre S.<sup>1</sup>, Seizova-Cajic T.<sup>1</sup>, Holcombe A.O.<sup>1</sup> and Birznieks I.<sup>2</sup><sup>1</sup>University of Sydney, NSW, Australia. <sup>2</sup>Prince of Wales Medical Research Institute, NSW, Australia.

Sustained observation of a moving surface causes adaptation in the human visual system, such that subsequently observed motion appears to be slower. Our goal was to explore the possibility of the same phenomenon of speed adaptation in the tactile domain. To create tactile motion, we used ridged, rotating drums on which participants rested their fingers. After adapting to motion applied to one hand, participants ( $n = 6$ ) judged the speed of a test stimulus that was a) the same direction as the previously exposed adapting stimulus, and b) the opposite direction to the adapting stimulus. Perceived speed was equally reduced following adaptation in both conditions. That is, the surface appeared to be slower regardless of the direction of the adapting motion. This result contrasts with those from visual experiments, where the direction of the adapting stimulus affects the perceived speed of the test stimulus. The lack of direction selectivity in our results suggests that tactile motion is processed quite differently than is visual motion. The reduction in perceived speed that we observed might be due to adaptation in temporal frequency channels.

## POS-TUE-167

**THE EFFECT OF CONTOUR LENGTH ON THE PERCEPTION OF LOCAL PERTURBATIONS**

Contini E.W. and McDonald J.S.

School of Psychology, The University of Sydney, NSW 2006 Australia.

It is thought the visual system evolved to optimally process contours (Geisler, Perry, Super & Gallogly, 2001) as well as informative contour irregularities (e.g. junctions and changes in orientation) which are frequently present in natural scenes, (Elder & Goldberg, 2002). However, relatively little is known about how context affects the perception of these irregularities. Here we investigate the effects of contour length on the perception of local contour irregularities. Experiment 1: 12 participants matched the amplitude of a sinusoidal line perturbation of an isolated test-segment to the perturbation amplitude of a match-segment embedded in contours of different predetermined lengths. Embedding the match segment in extended contours was found to cause systematic overestimation of perceived perturbation amplitude ( $p < .05$ ). Experiment 2: In a 2-alternative-forced-choice task, 15 participants judged whether very small segment perturbations of different amplitudes bowed leftward or rightward of absolute vertical. The task was performed for isolated segments and segments embedded in an extended contour. Surprisingly, detection of the perturbation direction was found to be better for isolated segments than embedded segments ( $p < .05$ ). The results from Experiment 1 can be explained with a gain control mechanism model similar to that which has been previously proposed to account for the tilt illusion (Schwartz & Simoncelli, 2001). However, Experiment 2 cannot be easily reconciled with this explanation. We discuss the results of Experiment 2 in terms of local versus global processing and relate both experiments to optimal processing.

## POS-TUE-166

**AUDITORY EVOKED POTENTIALS IN THE RAT AND THE "TWO-HIT" HYPOTHESIS OF SCHIZOPHRENIA**Nakamura T.<sup>1,2</sup>, Michie P.T.<sup>1,2</sup>, Fulham W.R.<sup>1,2</sup>, Hunter M.<sup>1</sup>, Budd T.W.<sup>1,2</sup>,Schall U.<sup>1,2</sup>, Kemp A.J.<sup>1</sup>, Cooper G.<sup>1,2</sup>, Todd J.<sup>1,2</sup> and Hodgson D.M.<sup>1,2</sup><sup>1</sup>The University of Newcastle, University Drive, Callaghan NSW 2308 Australia. <sup>2</sup>Schizophrenia Research Institute, 405 Liverpool Street, Darlinghurst NSW 2010 Australia.

Reduced mismatch negativity (MMN) is a robust finding in schizophrenia. It is correlated with other cognitive impairments in patients and with grey matter loss in frontotemporal regions. The goal of the present project is to establish a rodent model based on the "two-hit" hypothesis of schizophrenia and assess the success of the model by the extent to which it produces a reduction in MMN. However, the first step is to assess whether MMN-like activity in the rat meets criteria for identification as MMN, namely, sensitivity to probability effects. Event related potentials to 3 kHz tones were measured over auditory cortex in a freely moving animal ( $n = 1$ ). The stimulus paradigms allow (1) control for stimulus attributes and extraction of long (100 ms) and short (50 ms) duration MMN, (2) the effects of deviant probability on long duration MMN and (3) investigation of other auditory evoked potentials (AEPs). Preliminary data indicate a negativity around 50 ms after the offset of the longer duration deviant (1600 standard and 400 deviant trials). In addition, we observed four AEPs in non-deviant ERPs from 4800 trials: P10 (latency = 8 ms), N17 (22 ms), P23 (29 ms) and N38 (37 ms). While the preliminary data are encouraging, data need to be collected from more animals.

## POS-TUE-168

**A COMPARISON OF NEURONAL AND BEHAVIOURAL DETECTION THRESHOLDS IN RAT WHISKER-BARREL SYSTEM**

Adibi M., Cooper L. and Arabzadeh E.

School of Psychology, University of New South Wales.

A fundamental goal of systems neuroscience is to identify the link between neuronal activity and behaviour. The present study employed an animal model the rat whisker-barrel system – to compare the perceptual detection thresholds with the neuronal detection thresholds for a set of vibro-tactile stimuli. The whisker-region of rat somatosensory cortex (barrel cortex) is well-suited for examining the brain's encoding and decoding mechanisms due to its functional efficiency and its well-established anatomic and physiological organization. Here, we delivered sinusoidal whisker vibrations (frequencies of 30 to 100Hz with increment steps of 10Hz; amplitudes of 7.6, 15.2, and 30.4 $\mu$ m) to anaesthetised rats ( $n=6$ ) while recording extracellularly from barrel cortex neurons. A second group of rats ( $n=4$ ) were trained in a behavioural licking paradigm where a plate generated whisker vibrations at varying intervals and water became available at a spout 400ms after the onset of each vibration. The licking-rate during this stimulus presentation phase, prior to reward delivery, was taken as a response in anticipation of reward. As rats learned to detect vibrations, the amplitude and frequency was reduced to measure the behavioural detection thresholds. Our results confirmed that cortical activity encoded the product of amplitude and frequency which is proportional to the mean vibration speed. Neuronal response (spike rate) and behavioural response (licking rate) were both well-fitted by sigmoid curves (cumulative Gaussian). However, neuronal response profiles typically indicated a higher sensitivity compared to the behavioural detection thresholds. Although the lowest intensity stimulus was detected by most of the recorded neurons, none of the rats showed an enhanced licking response to this stimulus. These results suggest a superiority of performance for single cortical neurons over behaviour.



## POS-TUE-169

## DIFFERENTIAL CORTICAL REPRESENTATION OF INPUTS ARISING FROM THE UPPER ARM

Chelvanayagam D.K., Nagi S.S. and Mahns D.A.  
School of Medicine, University of Western Sydney, Sydney, NSW 1797, Australia.

**Purpose:** Peripherally-induced pain has a range of qualities that depend on the tissue stimulated. Cutaneous pain is often described as a sharp and/ or burning, well-localised sensation, whereas muscle pain has a dull-aching quality that is poorly localised. Intriguingly, bone pain shares qualities of cutaneous and muscle pain. **Methods:** Three 21-element platinum electrode arrays (provided by Cochlear Ltd.) were used to record the responses of the primary somatosensory cortex to electrical stimulation of the median nerve, the nerve innervating the triceps and/ or biceps muscles and the bone nerve entering the nutrient foramen of the humerus in anaesthetised rabbits (70mg.kg<sup>-1</sup>  $\alpha$ -chloralose, n=5). Electrical stimuli (1mA, 2ms) were applied to each nerve at an inter stimulus interval of ~2sec. **Results:** Cortical representation of bone and muscle inputs was contained within an area identified by stimulation of the median nerve. The positive-going cortical potentials tended to cluster on electrodes around distinct loci. The amplitude and extent of cortical activation varied between inputs from bone and muscle nerves relative to the median nerve (latency 13ms, amplitude 750 $\mu$ V). Bone-evoked cortical responses were observed in the majority of active recording electrodes identified by stimulating the median nerve and were of comparable amplitude but of longer latency (14ms). In contrast, stimulation of the muscle nerve either failed to evoke a cortical response or evoked responses but on fewer electrodes. Furthermore, the responses were of smaller amplitude and longer latency (17ms). **Conclusion:** Consistent with perceptual differences experienced during muscle and bone pain, a more limited pattern of cortical activation was observed following muscle stimulation.

## POS-TUE-170

## RESPONSES IN PRIMARY AND SECONDARY SOMATOSENSORY CORTICAL REGIONS IN THE CAT TO DUAL FREQUENCY VIBROTACTILE STIMULI APPLIED TO THE GLABROUS SKIN OF THE FOREPAW

Chen S.C.<sup>1,2</sup>, Byrnes-Preston P.J.<sup>1</sup>, Vickery R.M.<sup>1</sup>, Lovell N.H.<sup>1</sup> and Morley J.W.<sup>1,2</sup>  
<sup>1</sup>University of New South Wales, Sydney, Australia. <sup>2</sup>University of Western Sydney, Sydney, Australia.

The relative roles of the primary (SI) and secondary (SII) somatosensory regions of cortex in processing vibrotactile stimuli of high and low frequency remain unclear. To investigate this issue further we recorded the spatio-temporal patterns of activation of neurons in the cat SI and SII using multichannel electrodes and data acquisition. Sinusoidal vibrotactile stimuli of 20Hz, 200Hz or combined 20/200Hz were presented at various amplitude combinations to the glabrous skin of the forepaw of anaesthetized cats (n=4). Multi-unit spike activity was recorded from penetrating multi-electrode arrays (100-channel Utah or 64-channel NeuroNexus arrays) inserted into contralateral SI and SII, in each case in the region receiving input from the glabrous skin of the forepaw. The responses recorded in many cases were tuned specifically to either 20 or 200 Hz. However, in ~25% of the responsive electrode sites there was an inhibition of the response to the combined sinusoidal stimulation. In these cases, spiking activity was principally driven by either the 20 or 200 Hz stimulus, and as the amplitude of the other stimulus frequency was increased, the spike count reduced. In ~10% of responsive sites, the dual frequency stimulus produced spike counts greater than the linear summation of spiking activity to the individual frequencies. Inhibitory responses were more common in SI than SII, which is consistent with previous reports that SII provides inhibitory feedback to SI.

## POS-TUE-171

## A HIGH THROUGHPUT ASSAY TO MEASURE SENSORIMOTOR GATING (PPI) IN DROSOPHILA

Van Alphen B.<sup>1</sup>, Burne T.H.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup>, Mattingley J.<sup>1</sup>, McGrath J.J.<sup>1,2</sup> and van Swinderen B.<sup>1</sup>  
<sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia. <sup>2</sup>Queensland Centre for Mental Health Research, Wacol, QLD 4076 Australia.

**Introduction** In every day life, sensory systems are exposed to an incredible amount of information. To protect the brain from being overwhelmed, this information is filtered so only relevant information gets processed and transformed into action. Sensorimotor gating is one such filter mechanism. It refers to the state-dependent regulation of transmission, or gating, of sensory information to motor systems. Impairments in sensorimotor gating, such as pre-pulse inhibition (PPI), have been described in several psychiatric disorders like schizophrenia, autism and ADHD. **Methods** We study sensorimotor gating in *Drosophila* with a high-throughput behavioral paradigm that allows us to measure motor responses to moving visual stimuli in walking fruit flies. Groups of 25-30 flies are loaded into a maze placed over a screen showing moving visual stimuli. Flies move through the maze and pass eight successive left/right choice points after which they emerge in one of nine end tubes where they are counted. The average response is the Optomotor Index (OI). We measured the effect of briefly flashed visual stimuli on OI in two wildtype *Drosophila* strains: *Canton-S* and *Berlin*. **Results** Flash duration varied from 13-106 ms (n=240 per condition). In both strains, only 40 ms black flashes against a green background significantly reduced OI. Additionally, fly behavior was recorded using custom-made fly-tracking software. During 40 ms black flashes, behavior changed considerably. Flies spend longer in the maze and backtracked more. **Conclusions** We are currently investigating whether this behavioral change can be suppressed with a low intensity visual pre-pulse, in order to create a model of PPI in the fly. The development of such a model could be of enormous use given PPI deficits are considered a viable endophenotype of schizophrenia.

## POS-TUE-172

## PHYSIOLOGICAL NOISE IN THE FIRING OF HUMAN MUSCLE SPINDLE AFFERENTS

Birznies I.<sup>1</sup>, Boonstra T.W.<sup>2</sup> and Macefield V.G.<sup>1,3</sup>  
<sup>1</sup>Prince of Wales Medical Research Institute, Sydney, Australia. <sup>2</sup>The Black Dog Institute, Sydney, Australia. <sup>3</sup>School of Medicine, University of Western Sydney, Sydney, Australia.

Depending on their proximity to blood vessels some muscle spindle afferents may be driven by arterial pulsations. We explored whether a subtle cardiac modulation could be discerned in the background discharge of spontaneously active muscle spindles, which could contribute to their overall discharge variability and hence their capacity to encode length changes. Recordings were made from 11 primary and 6 secondary muscle spindle afferents innervating the pretibial flexors via microelectrodes inserted into the common peroneal nerve of awake human subjects. Pulse-related modulation of discharge was observed in 7 primary and 3 secondary afferents. On average, arterial pulsations could explain 17% (range 4-47%) of the total variance in muscle spindle discharge. The largest modulation within the cardiac cycle was observed ~300 ms after the R-wave. The size of the pulse wave modulation correlated neither with mean discharge rate, overall discharge variance nor pulse pressure (p>0.05; Spearman's rank correlation). To identify common features of modulation we used principal component analyses. Most of the modulatory pattern features were explained by the first principal component (p<0.05): 53% of the modulatory effect was accounted for by a pattern that shared common features between different afferents. The second principal component explained 21% of the variance; this was a modulation of the first mode that captured latency differences across afferents. The eigenvector coefficients indicated that the arterial pulse wave could show inhibitory as well as excitatory effects on discharge variability. We conclude that the spontaneous discharge of human muscle spindle afferents contains significant physiological noise that is determined primarily by mechanical disturbances by local blood vessels.

## POS-TUE-173

**REACTION TIMES IN AN ATTENTION TASK IS SHORTENED BY MICROSTIMULATION IN POSTERIOR PARIETAL CORTEX**

Levichkina E.<sup>1</sup>, Maloney R.<sup>1</sup>, Jayakumar J.<sup>1</sup>, Goodwin A.W.<sup>2</sup> and **Vidyasagar T.R.**<sup>1,2</sup>

<sup>1</sup>Department of Optometry & Vision Sciences, University of Melbourne, Victoria. <sup>2</sup>Dept of Anatomy & Cell Biology, University of Melbourne, Melbourne, Victoria.

Electrophysiological studies in macaques have shown attention related responses in a part of the posterior parietal cortex (lateral intraparietal area or LIP), but it is not known whether such neuronal activity affects decision making by the monkey. To demonstrate any causal relationship that may exist between LIP neurones and spatial attention, we applied brief pulses of a biphasic current to a small region of the LIP during a specific period of an attention-demanding delayed match to sample memory task. The task involved the monkey covertly attending to two grating patches presented sequentially for 100 msec each with a delay of 600 msec between them and matching the visual field location and orientation of the two gratings. We reported earlier that LIP neurones show increased coherence and response around the time of presentation of the second grating when the two gratings have the preferred orientation and location (Saalmann et al., Science, 316, 1612-1615, 2007). In the present study, we applied in one monkey microstimulation (20-50 microamp 200 microsecond pulses at 200 Hz) for 200 msec, starting 100 msec before the second grating. In match trials, when both stimuli were presented at the visual field location represented by the site of the stimulating electrode, reaction times were significantly shorter with microstimulation (n= 7 sessions; Paired t test, p=0.007). In trials with stimuli at visual field locations away from the stimulating electrode, there was no difference between unstimulated and stimulated trials (n=7; p=0.90). These results support the idea that activity in LIP may be causally related to focal spatial attention.

## POS-TUE-175

**GENETIC CONTRIBUTION TO INDIVIDUAL VARIATION IN BINOCULAR RIVALRY RATE**

**Miller S.M.**<sup>1</sup>, Hansell N.K.<sup>2</sup>, Ngo T.T.<sup>1</sup>, Liu G.B.<sup>3</sup>, Pettigrew J.D.<sup>4</sup>, Martin N.G.<sup>2</sup> and Wright M.J.<sup>2</sup>

<sup>1</sup>Perceptual and Clinical Neuroscience Group, SPPPM, Monash University, Melbourne, Australia. <sup>2</sup>Queensland Institute of Medical Research, Brisbane, Australia. <sup>3</sup>Dept Biological and Physical Sciences, Centre for Systems Biology, University of Southern Queensland, Toowoomba, Australia. <sup>4</sup>QBI, University of Queensland, Brisbane, Australia.

Binocular rivalry (BR) occurs when conflicting images are presented in corresponding locations of the two eyes. Perception alternates between the images at a rate that is relatively stable within individuals but that varies widely between individuals. The determinants of this variation are unknown. In addition, slow BR has been demonstrated in bipolar disorder, a psychiatric condition with high heritability (Pettigrew & Miller, 1998, Proc Roy Soc 265:2141-2148; Miller et al., 2003, Psychol Med 33:683-692; Nagamine et al., 2009, Bipolar Disord 11:539-546). The present study therefore examined whether there is a genetic contribution to individual variation in BR rate. We employed the twin method and studied both monozygotic twins (MZ; genetically identical; N=128 pairs) and dizygotic twins (DZ; who share roughly half their genes; N=220 pairs). Twin correlations for BR rate were 0.51 and 0.19, respectively. Genetic modeling showed 52% of the variance in BR rate was accounted for by additive genetic factors. In contrast, non-shared environment accounted for only 18%, with the remaining variance due to measurement error. This is the first study to report such a finding for BR and is also, to our knowledge, the first large study to show a substantial genetic contribution to individual variation in any post-retinal visual processing phenomenon (Wilmer, 2008, Spat Vis 21:561-579). The results suggest vigorous pursuit of genetic and molecular studies of BR, and further characterisation of slow BR as an endophenotype for bipolar disorder.

## POS-TUE-174

**STEADY-STATE VISUALLY EVOKED POTENTIALS (SSVEPS) REVEAL ATTENTION-LIKE BEHAVIOUR IN THE FRUIT FLY *DROSOPHILA MELANOGASTER***

**Van Swinderen B.** and Paulk A.C.

Queensland Brain Institute, University of Queensland.

Introduction: Correlates of visual selective attention can be measured in human EEG when subjects are presented with competing visual stimuli tagged by distinct flickering frequencies ("frequency tags"). Typically, transitions in attention are associated with changes in amplitude and coherence in the induced waveforms. How such changes relate to endogenous attentional mechanisms remains unclear. In order to investigate visual attention in a reductionist model, we have developed an SSVEP paradigm in the fruit fly, *Drosophila melanogaster*. Methods: Local Field Potentials (LFPs) were recorded from the brains of tethered flies presented with competing flickering images on a surrounding LED arena (n > 30 flies). Spectral analyses of fly brain activity were contrasted for frequency-tagged visuals. Results: We found central brain responses to different objects flickering simultaneously at distinct frequencies. These responses displayed temporal dynamics characteristic of attention-like states, notably when salience (novelty or heat) was associated with one or the other flickering object. In general, the amplitude of the more salient (i.e., attended) frequency was increased, and coherence between the attended frequency and endogenous 20-30 Hz oscillations was transiently increased as well. Data from training experiments (n = 10 flies) revealed that operant learning could be restricted to fly brain activity in response to competing visual objects. Conclusion: Our *Drosophila* SSVEP model offers a powerful approach to dissect fundamental mechanisms of visual selective attention. To more thoroughly explore visual selection and suppression effects in this small brain, we have developed a method of recording from multiple sites throughout successive layers of visual processing in the fly. Together with genetic tools, this strategy should help uncover the neuroanatomy responsible for attention-like processes in the fly brain.

## POS-TUE-176

**THE ROLE OF NEURAL SYNCHRONIZATION IN HIERARCHICAL MOTOR CONTROL**

**Boonstra T.W.**<sup>1,2</sup>, Daffertshofer A.<sup>3</sup> and Breakspear M.<sup>1,2,4</sup>

<sup>1</sup>School of Psychiatry, University of New South Wales, Sydney, Australia. <sup>2</sup>Black Dog Institute, Sydney, Australia. <sup>3</sup>Research Institute MOVE, VU University Amsterdam, The Netherlands. <sup>4</sup>Queensland Institute of Medical Research, Melbourne, Australia.

Neural dynamics display oscillatory activity at multiple spatial and temporal scales. Oscillations at large scales are due to synchronized activity in and between smaller subsystems and reflect a reciprocal relationship between such interactive parts and the collective pattern. These interactions can extend over several scales, giving rise to so-called nested oscillations such as phase-amplitude coupling between theta and gamma oscillations. In the present study, we sought to characterize nested oscillations during coordinated, rhythmic motor behavior in two experimental conditions. The first experiment comprised a bimanual tapping task in which participants (n=9) learned to perform a 5:3 polyrhythm during the acquisition of MEG and EMG data. Time-frequency analyses revealed distinct patterns of corticocortical and corticospinal synchronization. Specifically, neural activity between bilateral motor cortices was synchronized at approximately 30 Hz, whereas corticospinal activity was synchronized at 20-25 Hz. Both synchronization patterns exhibited event-related modulations such that the envelope was coupled to the force output of the contra-lateral finger. In the second experiments, EMG data were acquired whilst participants (n=10) tracked a sinusoidal target signal with their centre of gravity. Bilateral EMG coherence between homologous leg muscles was found to occur in two distinct frequency bands. Again, synchronization was not constant but modulated within a movement cycle and followed the time course of the activation patterns of the muscles. These nested synchronization patterns provide a framework for hierarchical motor control in which large-scale oscillations impose coordinated top-down influences that modulate neural processing and organize motor commands.

## POS-TUE-177

**DISCRIMINATION OF COMPLEX FORM BY SIMPLE OSCILLATOR NETWORKS**Maddess T.<sup>1</sup>, Taylor R.R.L.<sup>1</sup>, Lo Y.-W.<sup>1</sup> and Nagai Y.<sup>2</sup><sup>1</sup>ACEVS & CVS, Australian National University. <sup>2</sup>Center for Information Science, Kokushikan University.

**Purpose:** Natural images are rich in higher order spatial correlations. Brain scanning, psychophysics and electrophysiology indicate that humans are sensitive to these image properties. Isotrigon textures are useful for studying this sense. Like natural images these textures have low dimensionality (entropy) relative to random images, but like random images contain no average structure in their first to third order correlation functions. Thus, the structured appearance of these textures, and our ability to discriminate them from random textures, results from higher order spatial correlations. Recursive nonlinear processing can generate the higher order products of higher order correlations. We therefore examined whether small recursive oscillator networks could produce isotrigon texture discrimination performance that matches that of humans. **Methods:** The 23 subjects discriminated random textures from 53 isotrigon texture types. A range of network types were examined, all of which had a pooling readout oscillator. Differences in readout oscillator activity for isotrigon and random textures were measured. The inputs to the networks were small spatial receptive fields (RFs). We have shown that the entropy of textures sampled by these RF shapes also predicts human discrimination performance [Taylor et al. 2008. J Vision 8: 1-13]. The input oscillators are of a novel cubic form. **Results:** The two best network types found contained as few as 4 oscillators. Network activity matched human performance reasonably well,  $r^2$  up to 0.81  $\pm$  0.13 SE, even when the network parameters were fixed for all 53 texture types. The best RFs matched those of our entropy study. The networks resemble published models of illusory contour tours in V1 and V2, where isotrigon discrimination occurs. **Conclusions:** Overall it appears that relatively simple, short range, and biologically plausible, recursive processing could provide the basis for discrimination of complex form.

## POS-TUE-178

**LEARNING AND MEMORY PATHWAYS IN THE DROSOPHILA MUSHROOM BODIES AFFECT MOTION RESPONSE TO VISUAL STIMULI**Evans O., Paulk A. and Van Swinderen B.  
Queensland Brain Institute, The University of Queensland.

**Introduction:** Attention-like processes modulate behavioural responses to visual stimulation. Here, we use the *Drosophila* model to characterize visual behaviour and dissect its neural correlates. **Methods:** We developed a high-throughput paradigm that scores responsiveness of fruit flies to a variety of visual stimuli. Flies were exposed to visual stimuli while progressing through mazes overlaid on top of CRT monitors. Upon completion of the maze, flies emerged at the exit points and were automatically counted, after which an average response, the optomotor index, was calculated for each experiment. **Results:** Wild-type flies and *dunce*<sup>1</sup>, a learning and memory mutant, were used to evaluate this automated paradigm. *dunce*<sup>1</sup> mutants responded more strongly than wild type flies to moving stimuli across a wide range of luminosity, contrast, spatial, and temporal frequency values ( $p < 0.05$ ,  $n = 192$ ). To further dissect visual motion responses in *dunce*<sup>1</sup> mutants, we introduced conflicting visual stimuli. *dunce*<sup>1</sup> mutants retained a higher response than wild-type flies even when competing stimuli were occluding most of the field of view ( $p < 0.05$ ,  $n = 202$ ). Since *dunce* is strongly expressed in the mushroom bodies, the learning and memory brain structures, we hypothesized that they are involved in producing these optomotor responses. Expressing wild-type *dunce* cDNA in the mushroom bodies rescued the visual response to wild-type levels. Conversely, silencing the synaptic output from the mushroom bodies produced *dunce*-like visual responses. **Conclusion:** We have developed an efficient new method of screening visual phenotypes in *Drosophila*, and our results suggest that the mushroom bodies, structures previously associated with olfactory learning memory, are also involved in modulating visually driven behaviour in flies.

## POS-TUE-179

**THE CORRIDOR TASK AS A SENSORIMOTOR TEST FOR 6-OHDA LESIONED MICE**Fernando C.V., Parish C.L., Horne M.K. and Thompson L.  
Florey Neuroscience Institutes, The University of Melbourne, Parkville, Victoria, Australia.

The midbrain dopamine cell loss associated with Parkinson's disease is frequently modelled by unilateral 6-hydroxydopamine (6OHDA) injections into the nigrostriatal pathway resulting in side-biased motor impairments including akinesia, sensorimotor neglect and rotational behaviour. While behavioural tests for these impairments have been well established for rats, many are not well translated using mice. The corridor task is a drug free behavioural test that has been shown to detect lateralised neglect in 6OHDA lesioned rats. In the present study adult female Swiss mice ( $n = 51$ ) with large 6OHDA unilateral SNpc lesions were subjected to the corridor task to determine whether it could also be a suitable sensorimotor test in mice. The corridor task involved placement of food rewards at regular intervals along the left and right sides of a long narrow corridor. The number of retrievals made from the left and right side within 5 minutes were counted. The cylinder test and amphetamine induced rotational behaviour was also conducted to confirm and compare the validity of the corridor test with established behavioural tests. The corridor test revealed contralateral sensorimotor neglect in 98% of mice whilst only 82% mice responded to amphetamine induced rotations. The cylinder test did not show a significant impairment in the contralateral forelimb. Histological examination revealed that performance in the corridor test correlated with the loss of DA neuron - a discrepancy that commonly exists when comparing with rotational behaviour. Our findings indicate that the corridor task may be a sensitive test of lateralised sensorimotor response selection in mice and possibly a more reliable test to assess loss of midbrain dopamine neurons compared to amphetamine induced rotations and the cylinder test.

## POS-TUE-180

**STRUCTURE AND PUTATIVE FUNCTION OF ANTENNAL SENSILLA OF WORKERS OF COPTOTERMES FORMOSANUS SHIRAKI**Yanagawa A.<sup>1,3</sup>, Yoshimura T.<sup>1</sup>, Tsunoda K.<sup>1</sup>, Imamura Y.<sup>1</sup> and Yokohari F.<sup>2</sup><sup>1</sup>Research Institute for Sustainable Humanosphere, Kyoto University, Uji 611-0011, Japan. <sup>2</sup>Division of Biology, Department of Earth System Science, Faculty of Science, Fukuoka University, Fukuoka 814-0180, Japan. <sup>3</sup>Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

The difficulty in controlling termites by fungi as a biological agency is often dependent on the ambiguousness of their habitat and behavior. Therefore fundamental studies are needed to elucidate the nature of termites. A better understanding of the antennal sensory system, which is considered important in communication among termite individuals, will be helpful in developing fungal control of termites. Although early studies reported the external and ultrastructures of sensilla on the termite antennae, the number and distribution of sensilla on a whole antenna were not detailed yet. We categorized the antennal sensilla of *Coptotermes formosanus* Shiraki into nine types on the basis of the external structures of the cuticular apparatus of sensilla, and precisely examined their distribution by scanning electron microscopy. The classified antennal sensilla were chaeticum I, II and III, s. trichodeum I and II, s. basiconicum, s. capitulum, s. campaniformium and marginal sensillum. The morphological features and functions of these sensilla were also discussed in consideration of their structural and distributional characteristics.



## POS-TUE-181

**RE-EXAMINING PREVIOUSLY 'ESTABLISHED' NEURAL PATHWAYS USING CLASSICAL AND NOVEL METHODS**

**Turker K.S.<sup>1</sup>**, Binboga E.<sup>1</sup>, Prasartwuth O.<sup>2</sup>, Kahya M.C.<sup>1</sup>, Atis E.S.<sup>1</sup>, Ugincius P.<sup>3</sup>, Yavuz S.U.<sup>1</sup>, Sendemir-Urkmez A.<sup>1</sup>, Rogasch N.C.<sup>1</sup> and Burne J.A.<sup>4</sup>

<sup>1</sup>Ege University, Izmir, Türkiye. <sup>2</sup>Chiangmai University, Chiangmai, Thailand. <sup>3</sup>Kaunas University, Kaunas, Lithuania. <sup>4</sup>Sydney University, Sydney, Australia.

Intracellular recordings from regularly discharging motoneurons in brain slices recently showed that the classical methods (SEMG, PSTH) contain inherent errors (Türker and Powers, Trends Neurosci 2005). These errors are minimized using peristimulus frequencygram (PSF). We now aim to re-examine the synaptic connections in several human reflex pathways using PSF and to compare the results with classical methods in order to source possible errors. After obtaining informed consent from volunteers (n=47), wire electrodes were inserted into one of the following muscles: tibialis anterior, soleus, gastrocnemius, masseter and first dorsal interosseous. Subjects maintained constant discharge rates of selected motor unit while sensory afferents (spindle, tendon organ, mechanoreceptors) around these muscles were stimulated. The evoked responses were represented by the SEMG, PSTH and PSF. We found that the onset of inhibitory events was reliably determined from the reduction in spike counts in PSTH. However, the endpoints of the inhibitory periods were more optimally shown by the PSF compared with the other two methods ( $p < 0.001$ ). Similarly, the duration of excitation is better established using the PSF, as the falling phase of excitation appeared as an inhibition in the SEMG and PSTH (lower activity / spike counts). The PSF on the other hand showed this event as a continuation of excitation as the discharge rates remained above prestimulus levels. This study confirms the predictions from the slice work that the timing of synaptic events on motoneurons, is more optimally reconstructed by a combination of classical and PSF methods.

## POS-TUE-183

**FUNCTION OF EGF RECEPTOR SIGNALING IN THE NEONATAL HIPPOCAMPAL LESION MODEL OF SCHIZOPHRENIA**

**Mizuno M.<sup>1</sup>**, Sotoyama H.<sup>2</sup>, Namba H.<sup>2</sup> and Nawa H.<sup>1,2</sup>

<sup>1</sup>Center for Transdisciplinary Research, Niigata university. <sup>2</sup>Molecular Neurobiology, Brain Research Institute, Niigata University.

There are EGF and EGF receptor (ErbB1) abnormalities in brain tissues and blood of schizophrenic patients. Here we examined pathological roles of ErbB1 signaling in the schizophrenia model rats that are established with bilateral microinjections of ibotenic acid to the ventral hippocampus as neonates. Treatment with ibotenic acid altered HB-EGF and TGF alpha protein levels in the forebrain regions in parallel with persistent activation of ErbB1 receptors. On postnatal week 8, rats were tested for behavioral tasks. The hippocampus-lesion significantly impaired the behavioral scores of PPI and latent learning in adults. In context fear conditioned task, memory retentions were not affected although their latent learning was significantly disrupted. We determined the antipsychotic effects of several ErbB1 tyrosine kinase receptor inhibitors in this model. The abnormalities in PPI and latent inhibition were ameliorated by icv infusion of all the ErbB1 inhibitors from an osmotic pump. These results indicate that blockade of ErbB1 signals may be a novel antipsychotic target for schizophrenia and its related disorders.

## POS-TUE-182

**AN ASSOCIATION BETWEEN INEQUITY-AVERSE MORAL PREFERENCE AND RISK AVERSION IN DECISION-MAKING**

**Palmer C.J.<sup>1,2</sup>**, Paton B.<sup>1,2</sup>, Ngo T.T.<sup>1</sup>, Thomson R.H.<sup>3</sup>, Hohwy J.<sup>2</sup> and Miller S.M.<sup>1</sup>

<sup>1</sup>Perceptual and Clinical Neuroscience Group, SPPPM, Monash University. <sup>2</sup>School of Philosophy & Bioethics, Monash University. <sup>3</sup>MAPRC, Alfred Hospital and Monash University.

Neuroimaging studies of decision-making (DM) in moral and non-moral contexts have shown activation of insular and prefrontal cortex regions, implicating processes in common to both forms of DM. However, the relationship between an individual's moral and non-moral DM preferences has yet to be investigated. This study therefore examined whether moral preferences in a distributive justice task (DJT) are associated with preferences in financial risk-taking tasks. Twenty young adult participants completed a computerised DJT to assess their individual preferences for equity versus total material benefit when distributing meals amongst a group of disadvantaged children. Participants also completed three computerised financial gambling tasks (Iowa Gambling Task, Cambridge Risk Task, Balloon Analogue Risk Task) and a trait questionnaire (Dahlbäck Risk-Taking Propensity Scale) to assess their risky DM preferences. DJT inequity aversion was found to correlate significantly with risk aversion ( $r_s = .60$ ,  $p = .003$ ) when risk aversion was assessed by the Iowa Gambling Task, which contained uncertain potential outcomes, but not when risk aversion was assessed by the other measures. Inequity-averse distributive justice DM preferences therefore appear to be associated with risk-averse DM preferences when the potential outcomes of risky choices are uncertain. This finding suggests that factors contributing to individual differences in DM may generalise across moral and non-moral contexts, and is consistent with previous neuroimaging studies of insular and prefrontal cortex function during both types of DM.

## POS-TUE-184

**SPATIAL LEARNING-INDUCED INCREASE IN AGMATINE LEVELS AT HIPPOCAMPAL CA1 SYNAPSES**

**Leitch B.**, Shevtsova S., Reusch K., Bergin D.H. and Liu P.  
Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand.

Agmatine, a metabolite of L-arginine, is considered a novel putative neurotransmitter. Agmatine-like immunoreactivity has been detected in axon terminals that synapse with pyramidal cells of the hippocampus. However, the role of endogenous agmatine in learning and memory is poorly understood at present. Recent research has demonstrated water maze training-induced increase in the tissue concentration of agmatine in the CA1 sub-region of the hippocampus, a brain area that is critically involved in spatial reference memory. This finding suggests that endogenous agmatine may directly participate in learning and memory processes as a neurotransmitter. The aim of the present study was to further address this issue by investigating whether levels of agmatine are increased at synaptic terminals in the CA1 region following water maze training. Quantitative immunogold-labelling and electron-microscopical techniques were used to analyse agmatine levels in CA1 Schaffer collateral (SC) terminals (n=600) of rats that had been trained to find a hidden escape platform in the water maze task (WM, n=3), or forced to swim in the pool with no platform presented (SW, n=3). All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Agmatine levels were significantly increased in the synaptic terminals of SCs of trained WM group compared to the SW control group (all  $p < 0.001$ ). These results provide further evidence of the participation of endogenous agmatine in learning and memory processing.

## POS-TUE-185

# CAN RATS BE TRAINED TO DISCRIMINATE BETWEEN PRE- AND POST-SYNAPTIC SEROTONIN 1A RECEPTORS? INVESTIGATING THE EFFECTS OF NOVEL ANXIOLYTIC AND ANTIDEPRESSANT COMPOUNDS USING DRUG DISCRIMINATION

Vacy K., Ralph D., Tunstall B. and Broadbear J.  
School of Psychology, Psychiatry and Psychological Medicine,  
Monash University, Australia.

As a pharmacological target, the 5-HT<sub>1A</sub> receptor has shown considerable efficacy in clinical studies for the treatment of anxiety and depressive disorders. One explanation that has been proposed to account for the 2-4 week delay in the onset of therapeutic effects of antidepressants is that this is the time required for desensitization of pre-synaptic 5-HT<sub>1A</sub> receptors (autoreceptors). If true, it is likely that combining an autoreceptor-selective agonist with antidepressant treatment may lead to a more rapid onset of antidepressant or anxiolytic action. In this study we examined whether rats could be trained to report differences in the effects of a post-synaptic 5-HT<sub>1A</sub> selective agonist (F15599; 0.05-0.5mg/kg i.p.; 20 min ptt) and a pre-synaptic 5-HT<sub>1A</sub> selective agonist (F13714; 0.005-0.05mg/kg i.p.; 20 min ptt). Twenty-one (12 M, 9 F) Sprague-Dawley rats were trained to discriminate between the selective 5-HT<sub>1A</sub> agonist 8-OH-DPAT (0.1mg/kg i.p.; 20 min ptt) and its vehicle, saline. Both F15599 (n=8) and F13714 (n=8) fully substituted for the training drug. 8-OH-DPAT-appropriate responding for all three drugs was blocked by the selective 5HT<sub>1A</sub> receptor antagonist, WAY-100,635(n=4). There was a 20-fold difference in the doses at which F15599 (0.5mg/kg) and F13714 (0.025mg/kg) generalized to the 8-OH-DPAT training cue, supporting the conclusion that interoceptive cues produced by 8-OH-DPAT (0.1mg/kg) are mediated primarily through pre-synaptic 1A receptors. Preclinical evaluation of their antidepressant and anxiolytic utility will be the next important step in determining whether differences in their efficacy can be ascribed to actions at pre-synaptic, post-synaptic or the combination of these receptors.

## POS-TUE-187

# ALCOHOL REINSTATEMENT INDUCED NEURONAL ACTIVATION FOLLOWING PROTRACTED ABSTINENCE COMPARED TO REINSTATEMENT IMMEDIATELY FOLLOWING EXTINCTION

Jupp B.<sup>1</sup>, Krstew E.<sup>1</sup>, Dezsi G.<sup>1</sup> and Lawrence A.J.<sup>1,2</sup>  
<sup>1</sup>Florey Neuroscience Institutes, Parkville, Australia. <sup>2</sup>Centre for Neuroscience, The University of Melbourne, Parkville, Australia.

The enduring propensity for alcoholics to relapse even following years of abstinence presents a major hurdle for treatment. The neural mechanisms underlying continued susceptibility to relapse are largely unknown due to a lack of appropriate animal models to investigate relapse following extended abstinence. Here we report such a model and utilize it to investigate the pattern of neuronal activation during cue-induced reinstatement and following administration of the orexin-1 antagonist SB-334867 (SB). Rats were trained to self administer alcohol under an operant cue-conditioned paradigm then divided into two groups; early (reinstated immediately following extinction), and late (extinguished and then housed for five months before reinstatement). During reinstatement animals were treated with vehicle (early n=15, late n=9) or SB (20mg/kg ip; early n=12, late n=8). Fos expression was compared between each group and to animals who underwent extinction only (n=3). SB significantly attenuated reinstatement in both early and late groups. Early reinstatement increased Fos expression in the nucleus accumbens, the lateral and dorsomedial hypothalamus and bed nucleus, while late reinstatement further increased Fos expression in the accumbens shell, lateral hypothalamus, ventral bed nucleus and nucleus incertus. Activity in the basolateral and central amygdala was also increased late when compared to extinction. SB decreased Fos expression in the accumbens core in the early, but not late group. Cue-induced alcohol seeking can be triggered following protracted abstinence and involves a differential pattern of activation when compared to reinstatement immediately following extinction. SB was able to inhibit both early and late reinstatement and also demonstrated differential activation between the two paradigms.

## POS-TUE-186

# BEHAVIOURAL CORRELATES OF GESTATIONAL LOW DOSE ETHANOL EXPOSURE IN AGED OFFSPRING

Cullen C.L.<sup>1</sup>, Moritz K.M.<sup>1</sup>, Lavidis N.A.<sup>1</sup> and Burne T.H.J.<sup>1,2</sup>  
<sup>1</sup>School of Biomedical Sciences, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia 4072. <sup>2</sup>Queensland Brain Institute, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia 4072.

**Purpose:** Excessive alcohol consumption during pregnancy can lead to a wide spectrum of disorders and defects in offspring, which are collectively referred to as Foetal Alcohol Syndrome. However, little is known about the long term effects of mild alcohol consumption during pregnancy. The aim of this study was to examine the effect of exposure to a low dose ethanol diet during gestation on behavioural changes in aged adult offspring. **Methods:** Female Sprague Dawley rats were fed a liquid diet containing a low dose of ethanol (6% vol/vol, Ethanol) or a calorie matched control diet for the duration of pregnancy (Control). Male (n=14, Control; n=8, Ethanol) and female (n=10, Control; n=9, Ethanol) offspring were tested at 15-18 months of age to assess a number of behavioural domains including anxiety, exploration, sensorimotor gating and spatial memory, as well as ethanol preference. **Results:** Prenatal exposure to a low ethanol diet resulted in a subtle, however not significant (p=0.08), behavioural phenotype affecting aspects of anxiety and neophobia. However, there was no effect of prenatal treatment on locomotion, sensorimotor gating or spatial memory (p>0.05). Finally, the Control rats tended to have a greater preference for ethanol than did rats in the Ethanol group in a two bottle choice paradigm (p=0.06). **Conclusions:** Exposure to low dose ethanol during early neural development did not lead to long lasting behavioural changes in aged adult offspring. This indicates that a small amount of alcohol during pregnancy does not result in significant changes in foetal nervous system development.

## POS-TUE-188

# THE IMPACT OF STRESS ON A HETEROZYGOUS NEUREGULIN 1 MUTANT MOUSE MODEL

Chesworth R.<sup>1,2</sup> and Karl T.<sup>1,2,3</sup>  
<sup>1</sup>Prince of Wales Medical Research Institute. <sup>2</sup>Schizophrenia Research Institute. <sup>3</sup>University of New South Wales.

**Purpose:** A mouse model for the schizophrenia candidate gene neuregulin 1 (*NRG1*) [i.e. transmembrane domain *Nrg1* mutant mice (*Nrg1* HET)] exhibits a schizophrenia-related behavioural phenotype. *Nrg1* HETs are more susceptible to environmental factors such as cannabis (i.e. acute and chronic Δ-9-tetrahydrocannabinol exposure) or altered housing conditions (i.e. environmental enrichment). During animal transfers between laboratories/institutes, *Nrg1* mutant mice appear to be differentially affected by stress compared to their non-mutant littermates. **Methods:** We investigated the endocrine and behavioural response of male and female heterozygous *Nrg1* HET and their wild type-like (WT) littermates (n ≥ 10) after acute exposure to restraint or swim stress. Different sets of mice were tested in behavioural paradigms for locomotion, exploration and anxiety (e.g. open field, light dark, zero maze). Blood samples – collected at baseline and after stress exposure – were analysed for plasma levels of the stress hormone corticosterone. **Results:** WT and *Nrg1* HETs were susceptible to the locomotion-inhibiting and anxiety-elevating effects of restraint and swim stress. All test mice exhibited an endocrine stress response with corticosterone plasma levels being increased after stress induction. However, it was dependent on the stressor and the behavioural test paradigm used, whether *Nrg1* mutant mice showed an altered sensitivity to the behavioural effects of stress. The endocrine stress response of WT and *Nrg1* HET mice was identical. **Conclusion:** These data suggest that a variation in the *Nrg1* gene alters the sensitivity specifically to some but not all environmental manipulations/factors. In future, the *Nrg1* mouse model will be tested regarding its response to social stress.

## POS-TUE-189

**EXERCISE OR COMFORT FOOD REDUCES STRESS RESPONSE FOLLOWING PROLONGED MATERNAL SEPARATION IN RATS**

**Maniam J.** and Morris M.J.  
The University of New South Wales, Sydney.

Prolonged maternal separation (MS) causes HPA axis dysregulation with elevated plasma corticosterone (CORT), decreased glucocorticoid receptor (GR) mRNA expression and behavioural outcomes such as anxiety and depression-like behaviour. We investigated the effects of voluntary exercise and/or palatable high fat diet (HFD) following MS. Sprague-Dawley litters were assigned to brief (15 min, S15) or prolonged (180 min, S180) separation from postnatal day (PND) 2-14. At PND 21, males from each litter were assigned to either laboratory chow (11% fat) or HFD (32 % fat). Half the rats had continuous access to running wheels (voluntary exercise). Stress reactivity was measured at PND 60, using 15 min immobilization and sampling at 0, 15, 30, 45, 60 min for plasma CORT. Hippocampus was collected at 14 weeks for GR mRNA expression. A significant interaction between treatment and diet was observed for CORT ( $F=3.77$ ,  $p=0.02$ ). S180 rats on chow had increased basal plasma CORT and CORT response to stress (area under CORT curve 1.4 times higher than S15 rats, ANOVA,  $p<0.05$ ) and this was reduced by HFD consumption ( $t=3.37$ ,  $p<0.01$ ) or exercise alone ( $t=2.28$ ,  $p<0.05$ ). Combined exercise and HFD appeared to reduce plasma CORT however this was not significant ( $p=0.06$ ). Hippocampal GR mRNA was significantly reduced in S180 versus S15 rats ( $t=2.60$ ,  $p<0.05$ ). S180 rats subjected to exercise and HFD alone had increased GR mRNA compared to S180 chow ( $p<0.05$ ). Combining HFD and exercise did not alter expression of GR in S180 compared to chow fed rats. HFD or exercise blunted stress responsivity in S180 offspring and combining HFD and exercise had no added benefit. These effects appear to be mediated by increased hippocampal GR mRNA.

## POS-TUE-191

**ANXIETY AND CENTRAL RESPONSES TO RESTRAINT STRESS IN RATS MADE LEAN BY NEONATAL UNDERFEEDING**

**Spencer S.J.**, Bulfin L.J. and Tilbrook A.  
Department of Physiology, Faculty of Medicine, Monash University, Melbourne, Victoria, Australia.

Stress and the myriad diseases caused, triggered, or exacerbated by it costs countries millions of dollars annually and individuals their health and their lives. An excellent strategy to treat overactive and potentially dangerous responses to stress is to exploit the body's own inherent stress-inhibitory mechanisms. Stress responses can differ between individuals depending upon their level and distribution of adiposity, and we have recently shown that increased adiposity is associated with exacerbated responses to psychological stress. The converse may be true for lean animals. In this investigation we hypothesized that lean animals would have reduced anxiety and attenuated hypothalamic-pituitary-adrenal axis responses to psychological stress. Our findings show that rats made lean by being suckled in a large litter ( $n = 10$  males, females) show reduced levels of anxiety compared with those from normal litters ( $n = 10$  males, females) when tested in the elevated plus maze. These lean animals also have attenuated activation of the paraventricular nucleus of the hypothalamus in response to the psychological stress, restraint. Understanding the mechanisms by which these stress responses are attenuated in lean animals will be important for future strategies to treat overactive stress responses in humans.

## POS-TUE-190

**BEHAVIOURAL EFFECTS OF MATERNAL OBESITY: REDUCED ANXIETY IN FEMALE OFFSPRING OF OBESE MOTHERS**

**Morris M.J.**<sup>1</sup>, Hossain S.<sup>1</sup>, South T.<sup>1</sup> and Chen H.<sup>1,2</sup>  
<sup>1</sup>Department of Pharmacology, Medical Sciences, University of New South Wales, 2052. <sup>2</sup>Faculty of Science, University of Technology, 2007.

The obesity epidemic represents not only an immediate public health burden, but threatens future generations, as maternal obesity increases the risk of offspring obesity. We have shown that high-fat-diet consumption (HFD) after weaning exacerbates offspring metabolic risk. Obesity has been shown to influence behaviors such as learning, but data are conflicting. We tested the effects of maternal obesity on offspring anxiety levels, learning and memory. Female Sprague Dawley rats were fed chow (C) or HFD for 6 weeks before mating, throughout gestation and lactation. At 20 days, female pups were weaned onto either C or HFD, yielding CC, CH, HC and HH groups. Pups (12 per group) underwent anxiety testing (elevated plus maze, EPM), Y maze (spatial memory) and forced swim test (FST) from 9-19 weeks. Offspring of obese mothers (HC) were significantly heavier at weaning with increased adiposity and this was amplified by HFD (HH). At 11 weeks, HC and HH offspring spent more time, and made more entries, in the open arm of the EPM, with more exploratory behaviour assessed as head dips, compared to CC and CH offspring ( $P<0.05$ ). Postweaning diet had no significant impact. No difference was observed in time spent in the novel arm of the Y maze. While some effects of maternal diet were observed on FST, with increased immobility in offspring of obese mothers, this appeared to be related to overall adiposity. At 21 weeks leptin was markedly increased in HH versus HC offspring. Therefore, in females, maternal obesity appears to reduce offspring anxiety-like behaviour; ongoing work is aimed at dissecting underlying mechanisms.

## POS-TUE-192

**LYCIUM BARBARUM (WOLFBERRY) POLYSACCHARIDE FACILITATES EJACULATORY BEHAVIOR IN MALE RATS**

**Lau B.W.M.**<sup>1,2,3</sup>, Yau S.Y.<sup>1,2,3</sup>, Lee C.D.<sup>1,2,3</sup>, Chang R.C.C.<sup>2,3,4</sup> and So K.F.<sup>1,2,5</sup>  
<sup>1</sup>Department of Anatomy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China. <sup>2</sup>The State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China. <sup>3</sup>Research Centre of Heart, Brain, Hormone and Healthy Aging, Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China. <sup>4</sup>Laboratory of Neurodegenerative Diseases, Department of Anatomy, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China. <sup>5</sup>Joint laboratory for Brain function and Health (BFAH), Jinan University and The University of Hong Kong, GuangZhou, China.

**Objective:** Lycium barbarum (wolfberry) is a traditional Chinese medicine, which has been considered to have therapeutic effect on male infertility. However, there is a lack of studies support the claims. We thus investigated the effect of Lycium barbarum polysaccharide (LBP), a major component of wolfberry, on male rat copulatory behavior. **Method:** Sprague-Dawley rats were divided into two groups ( $n=8$  for each group). The first group received oral feeding of LBP at dosage of 1mg/kg daily. The control group received vehicle (0.01M phosphate-buffered saline, served as control) feeding daily for 21 days. Copulatory tests were conducted at 7, 14 and 21 days after initiation of treatment. **Results:** Compared to control animals, animals fed with 1mg/kg LBP showed improved copulatory behavior in terms of: 1. higher copulatory efficiency (i.e. higher frequency to show intromission rather than mounting during the test), 2. higher ejaculation frequency and 3. shorter ejaculation latency. The differences were found at all time points (Analyzed with two-tailed student's t-test,  $p<0.05$ ). There is no significant difference found between the two groups in terms of mount/intromission latency, which indicates no difference in time required for initiation of sexual activity. Additionally, no difference in mount frequency and intromission frequency was found. **Conclusion:** The present study provides scientific evidence for the traditional use of Lycium barbarum on male sexual behavior. The result provides basis for further study of wolfberry on sexual functioning and its use as an alternative treatment in reproductive medicine.



## POS-TUE-193

**ASSESSING THE ROLES OF OXYTOCIN AND ARGININE VASOPRESSIN**

**Mak P.** and Broadbear J.  
Monash University.

The neuropeptides oxytocin and arginine vasopressin are implicated in the regulation of depressive- and anxiety-like behaviours. Oxytocin is thought to promote anxiolytic and antidepressant-like effects while arginine vasopressin may increase anxious and aggressive behaviours. The present study examined the effects of the oxytocin agonist, carbetocin (2.5 and 5 mg/kg), and the vasopressin agonist, desmopressin (1 and 5 mg/kg) following intravenous administration, in a measure of depressive-like activity (forced swimming test; FST), and in two models measuring anxiety-like activity (elevated plus maze; EPM, and open field; OF). The oxytocin/AVP<sub>1A</sub> receptor antagonist, atosiban (1 mg/kg), was also investigated, both in isolation and in combination with carbetocin and desmopressin. Young adult male Sprague-Dawley rats (n=110) were tested once in each behavioural paradigm following these drug treatments and their effects compared with those of imipramine (a tricyclic antidepressant; 10mg/kg) and midazolam (a benzodiazepine; 1mg/kg). The results supported the hypotheses that carbetocin would have antidepressant-like effects in the FST, and that desmopressin would have anxiogenic-like effects in the EPM. Surprisingly, the oxytocin/vasopressin<sub>A</sub> antagonist atosiban produced anxiolytic effects in the EPM. As expected, the anxiogenic effects of desmopressin and antidepressant effects of carbetocin were both attenuated by coadministration with atosiban. A lack of anxiolytic-like effects of carbetocin and depressant-like effects of desmopressin strengthens the conclusion that central AVP<sub>1A</sub> receptors are selectively implicated in the anxiety response, while antidepressant-like effects are the domain of central carbetocin receptors.

## POS-TUE-194

**ADVANCED PATERNAL AGE IS ASSOCIATED WITH SPONTANEOUS HYPERLOCOMOTION AND ALTERATIONS IN RESPONSE TO AMPHETAMINE IN C57BL/6J MICE**

**Foldi C.J.<sup>1</sup>**, McGrath J.J.<sup>1,2</sup>, Eyles D.W.<sup>1,2</sup> and Burne T.H.J.<sup>1,2</sup>

<sup>1</sup>Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia. <sup>2</sup>Queensland Centre for Mental Health Research, Wacol, QLD 4076 Australia.

**Purpose:** Advanced paternal age (APA) is associated with an increased risk of neurodevelopmental disorders in offspring, including schizophrenia and autism. We have developed a mouse model to examine behavioural effects in offspring from older fathers (advanced paternal age, APA) compared with young fathers (young paternal age, YPA). The aim of the present study was to investigate the effects of APA on locomotion in both novel and home-cage environments and in response to d-amphetamine (AMPH) challenge. **Methods:** The adult offspring (N=123) of young (3 month-old, YPA) and old (12-18 month-old, APA) C57Bl/6J sires underwent a behavioural test battery comprising tests for baseline locomotion, anxiety and exploration. Offspring were then treated with AMPH and their locomotor response recorded in either a novel environment (automated open field) or in a familiar home-cage (PhenoTyper chamber). **Results:** Male APA offspring travelled significantly further than YPA males in the holeboard ( $p=.03$ ) and marble burying test ( $p=.01$ ) as well as following AMPH exposure in a novel environment, though this failed to reach significance ( $p=.12$ ). In the home-cage, male APA mice demonstrated decreased activity compared to controls, which was specific to the initiation of the dark phase of the cycle (7pm to midnight). There was no significant effect of paternal age on AMPH-induced locomotion in the home-cage. Behavioural changes in female APA offspring were restricted to their response to AMPH in the novel arena, in which APA females had transiently reduced locomotor scores compared with YPA females ( $p=.045$ ). **Conclusion:** Male APA mice demonstrated spontaneous hyperlocomotion and we show a variable response to amphetamine, dependent on sex and arena. This may implicate abnormalities in dopamine signalling however given the sex- and arena-specific nature of these findings the role of sex hormones and other neurotransmitter systems cannot be excluded. These results provide clear evidence that APA is associated with long-term behavioural changes, particularly in male offspring.

## POS-TUE-195

**THE EFFECTS OF OREXIN-1 RECEPTOR ANTAGONISM ON ALCOHOL SELF-ADMINISTRATION AND RELAPSE BEHAVIOUR IN RATS**

**Krivdic B.<sup>1,3</sup>**, Duncan J.R.<sup>1</sup>, Francis A.J.P.<sup>3</sup>, Krstew E.<sup>1</sup> and Lawrence A.J.L.<sup>1,2</sup>

<sup>1</sup>Florey Neurosciences Institutes, Parkville, Vic, 3010. <sup>2</sup>Centre for Neuroscience, University of Melbourne, Parkville, Victoria. <sup>3</sup>Division of Psychology, RMIT University, Bundoora, Victoria.

The neuropeptide orexin (also known as hypocretin) has been implicated in arousal, feeding and more recently in drug-seeking. The current study aimed to (1) investigate whether the selective orexin-1 receptor antagonist SB-334867 could reduce the motivation to self-administer alcohol and (2) examine cue-induced alcohol-seeking following prolonged abstinence in alcohol-preferring (iP) rats. Intra-peritoneal injections of SB-334867 or vehicle were administered prior to operant sessions under fixed ratio (n = 20) and progressive ratio (n = 18) schedules. Rats were then withdrawn from alcohol for 7, 14, 28 or 56 days, following which they were returned to the operant chambers and tested under extinction conditions in the presence of alcohol-related cues. Results indicated that SB-334867 significantly attenuated volitional alcohol self-administration ( $p<.05$ ) and also alcohol reward breakpoint under progressive ratio ( $p<.05$ ). These data suggest orexins may be implicated in both consummatory and appetitive responding for alcohol. Furthermore, rats displayed robust cue-induced alcohol seeking following all periods of abstinence and SB-334867 attenuated this response in rats abstinent for 14 days ( $p<.05$ ). These results further implicate orexin in the modulation of the rewarding effects of alcohol and the salience of alcohol-related cues.

## POS-TUE-196

**α4-S248F MICE SHOW INCREASED IV SELF-ADMINISTRATION OF LOW DOSE NICOTINE**

**Cahir E.**, Drago J. and Lawrence A.  
Florey Neuroscience Institutes, Parkville, Victoria.

**PURPOSE:** A role α4β2 nicotinic acetylcholine receptors in mediating the reinforcing effects of nicotine has been asserted. Mice with a point mutation of the α4 nicotinic subunit that confers an increased sensitivity to low doses of nicotine (α4-S248F mice) were assessed in comparison to wildtype littermates for intravenous self-administration of nicotine. **METHODS:** Mice to undergo surgery for operant intravenous self-administration (IVSA) were first trained to respond for sucrose with two levers (reward-paired and inactive) on an FR1 schedule of responding, with olfactory and visual reward-paired cues. Following insertion of a catheter into the jugular vein, mice were then allowed to respond for nicotine (0.03, 0.05 and 0.07 mg/kg/infusion). Mice were also assessed for progressive ratio responding and cue-induced drug seeking following a period of withdrawal for all doses. **RESULTS:** Mice established stable responding for nicotine across all doses. At the doses of 0.05 and 0.07 mg/kg/inf, no difference was observed between the response rates of each genotype, for acquisition and maintenance of responding, motivation for drug as measured by progressive ratio responding nor for cue-induced drug-seeking. However, when the dose was reduced to 0.03 mg/kg/inf of nicotine, α4-S248F mice showed increased responding compared to WT littermates across all measures, with mice earning significantly greater rewards, and showing increased motivation for drug in progressive ratio responding and cue-induced drug seeking. **CONCLUSION:** The increased rewarding and motivational effects of nicotine in α4-S248F mice at the low dose of nicotine supports a role for α4\* nicotinic receptors in mediating motivational and reinforcing properties of nicotine.

## POS-TUE-197

**MGLU5 RECEPTORS REGULATE OPIATE SELF ADMINISTRATION AND RELAPSE BEHAVIOUR IN MICE**Stagnitti M.R.<sup>1</sup>, Brown R.M.<sup>1,3</sup>, Duncan J.R.<sup>1</sup> and Lawrence A.J.<sup>1,2</sup><sup>1</sup>Florey Neuroscience Institutes, Uni. Melb., Parkville, Vic., 3010.<sup>2</sup>Centre for Neuroscience, Uni. Melb., Parkville, Vic., 3010. <sup>3</sup>Monash Institute of Pharmaceutical Sciences, Parkville, Vic., 3052.

Opiates represent the single largest contribution to illicit drug-related mortality and morbidity worldwide and effective treatment remains a major clinical problem. The metabotropic glutamate 5 (mGlu5) receptor has been implicated in drug induced plasticity and is believed to play a role in mediating the reinforcing properties of drugs of abuse. Previously we showed that selective antagonism of mGlu5 receptors with 3-[(2-methyl-1,3-thiazol-4-yl) ethynyl]-pyridine (MTEP) decreases ethanol self-administration, however the effect of MTEP on opiate self-administration has not been determined. Therefore we hypothesized that mGlu5 receptors play a role in mediating the self administration of opiates and contribute to relapse to opiate-seeking elicited by drug-associated cues. CD1 outbred wildtype mice were implanted with indwelling intravenous catheters and trained to self administer morphine (0.1mg/kg/infusion, i.v.) on a fixed ratio (FR)-1 schedule. After self-administration had stabilized, mice were treated with vehicle or MTEP (20mg/kg, i.p.) prior to an operant session in a repeated measures design. Mice readily acquired self-administration of morphine preferentially responding on the morphine-paired lever ( $p < 0.05$ ). Pretreatment with MTEP selectively reduced responding on the morphine paired lever ( $p < 0.05$ ). This corresponded with a  $>50\%$  reduction in total drug infusions ( $p < 0.05$ ). Responding on the morphine-paired lever returned to control levels the day after MTEP treatment. After 3 weeks withdrawal, mice were injected with vehicle ( $n=8$ ) or MTEP ( $n=7$ ) and returned to the chamber in the presence of drug-paired cues. MTEP also significantly reduced cue-induced opiate-seeking following abstinence ( $p < 0.05$ ). In conclusion we have shown that glutamatergic signaling via mGlu5 receptors plays role in regulating opiate self-administration and relapse to drug seeking behaviour.

## POS-TUE-199

**EVALUATING THE CONTRIBUTION OF SEROTONIN RECEPTOR SUBTYPES AND 'BINGE' MDMA EXPOSURE ON THE DISCRIMINATIVE STIMULUS EFFECTS OF MDMA IN RATS**Smithies V. and Broadbear J.  
Monash University.

3,4-Methylenedioxymethamphetamine (MDMA; 'Ecstasy') shares psychoactive effects with both the stimulant, amphetamine (dopaminergic) and the hallucinogen, LSD (serotonergic). The majority of MDMA's reported effects and toxicity have been linked to its actions on serotonergic neurotransmission. One way to study MDMA's serotonergic effects is to train rats to distinguish between dopaminergic stimulant effects and mood and perception-altering serotonergic effects using a three-way drug discrimination paradigm. Once male and female Sprague Dawley rats ( $n=18$ ) learned to reliably differentiate between amphetamine (0.75mg/kg), MDMA (1.5mg/kg) and saline, the contributions of serotonin<sub>1A</sub> (5-HT<sub>1A</sub>) and 5-HT<sub>2A/C</sub> receptors to MDMA's interoceptive effects were evaluated. This was done both before and after the rats were exposed to an MDMA 'binge' (3 x 10mg/kg at two hourly intervals) to determine whether a reportedly neurotoxic dosing regimen would disrupt the interoceptive cues of MDMA. Blockade of 5-HT<sub>1A</sub> or 5-HT<sub>2A/C</sub> receptors, via administration of WAY 100,635 (1 mg/kg) or ritanserin (1.5 and 3 mg/kg), significantly disrupted MDMA-appropriate responding. Binge administration resulted in selective disruption to the MDMA training cue in the majority of rats during the two subsequent weeks. Once the discrimination had recovered, repeating the antagonist tests revealed that the contributions of the 5-HT<sub>1A</sub> and 5-HT<sub>2A/C</sub> receptors to the MDMA discriminative cues were not significantly different to what was measured prior to the 'binge'. This study provides support for the importance of 5-HT<sub>1A</sub> and 5-HT<sub>2A/C</sub> mediated cues in the discriminative, and by extension behavioural and neurotoxic effects of MDMA, and suggests that its discriminative stimulus effects are only temporarily disrupted following high-dose MDMA exposure.

## POS-TUE-198

**THE EFFECT OF OXYTOCIN AND VASOPRESSIN ON METHAMPHETAMINE AND MDMA ('ECSTASY')-INDUCED REWARD IN RATS**Rourke P.I.<sup>1</sup>, Hunt G.E.<sup>2</sup>, McGregor I.S.<sup>3</sup> and Cornish J.L.<sup>1</sup><sup>1</sup>Department of Psychology, Macquarie University. <sup>2</sup>Discipline ofPsychological Medicine, University of Sydney. <sup>3</sup>School of Psychology, University of Sydney.

The neuropeptide oxytocin is increasingly recognized as modulating the rewarding effects of psychostimulants. Oxytocin is known for mediating the social rewarding effects of 3,4-methylenedioxymethamphetamine (MDMA) and we have recently shown that systemic oxytocin pretreatment reduces the motivation for rats to self-administer intravenous infusions of methamphetamine (METH). However a role for oxytocin in MDMA or METH-induced contextual reward has not yet been demonstrated in rats. In addition, an effect of the neuropeptide vasopressin on psychostimulant reward has been less well described. This study investigated the effect of pretreatment with oxytocin (600 µg/kg ip) or [Arg<sup>8</sup>]-vasopressin (3ng/kg ip) on MDMA (5mg/kg ip) or METH (1mg/kg ip) conditioned place preference (CPP) in male Sprague-Dawley rats. CPP was conducted using 6 daily conditioning sessions (30 min), where animals were paired to one context following drug administration for 3 sessions, alternated daily with 3 sessions of vehicle-paired context. Odour cues were paired to each context. In comparison to pre-conditioning, animals treated with METH spent significantly more time in the drug-paired context ( $n=10$ ) indicating a CPP to METH. Pretreatment with oxytocin ( $n=10$ ) or [Arg<sup>8</sup>]-vasopressin ( $n=9$ ) prior to drug conditioning sessions prevented METH-induced CPP. In comparison to pre-conditioning, animals treated with MDMA spent significantly more time in the drug-paired context ( $n=10$ ) indicating a CPP to MDMA. Pretreatment with oxytocin ( $n=10$ ) or [Arg<sup>8</sup>]-vasopressin ( $n=10$ ) did not prevent MDMA-induced CPP. These data show that both neuropeptides are involved in modulating the rewarding effects of METH but do not modulate MDMA-induced contextual reward.

## POS-TUE-200

**AGE-RELATED INCREASE IN MITOCHONDRIAL DNA DELETION MUTATIONS IN RAT BRAIN**Cahif A.<sup>1,2</sup>, Soliman M.<sup>1,2</sup> and Smith D.W.<sup>1,2</sup><sup>1</sup>School of Biomedical Sciences & Pharmacy, The Centre for Brain & Mental Health Research, University of Newcastle. <sup>2</sup>Hunter Medical Research Institute, NSW, Australia.

**AIMS:** Accumulation of mitochondrial DNA (mtDNA) mutations has been implicated in ageing. Consistent with this notion, studies in human brain show mtDNA deletions accumulate with age in a region specific pattern. The aims of the present study were to determine whether a similar phenomenon occurred in the brain of a commonly used animal model for ageing, the F344 rat. **METHODS:** DNA was extracted from cerebellum, midbrain, striatum and cerebral cortex of 6 young (4-6 months), 5 middle aged (12-15 months) and 6 old (24-27 months) F344 rats. Long PCR was carried out to amplify the major and minor arc regions of the mtDNA and reaction products separated by agarose gel electrophoresis. Quantification of mtDNA deletions was carried out using qPCR comparing the delta CT of a major arc gene, ND4, to that of a minor arc gene, 12S rRNA. **RESULTS:** Our preliminary long PCR results demonstrate a brain region-specific, age-related mtDNA deletion pattern, with the cerebellum being relatively spared and the striatum and cerebral cortex being most profoundly affected. The majority of deletions, in affected brain regions, were seen in the major arc of mtDNA with the minor arc being relatively free of deletions. These results are consistent with those previously reported for humans. The age-related mtDNA deletions apparent with long PCR, were confirmed by qPCR analysis which showed 28% and 25% increases in mtDNA deletion burden in the cerebral cortex and striatum, respectively, of old rats compared to young rats ( $p < 0.05$ ). **CONCLUSIONS:** These data indicate the rat is a suitable model for studying age-related mtDNA changes in the brain.

## POS-TUE-201

**CHARACTERISATION OF THE FOREBRAIN-NEURON SPECIFIC IRAP KNOCKOUT MOUSE**Yeatman H.R.<sup>1,2</sup>, Burns P.<sup>1</sup>, Albiston A.L.<sup>1</sup> and Chai S.Y.<sup>1,2</sup><sup>1</sup>Florey Neuroscience Institutes, Melbourne. <sup>2</sup>Centre for Neuroscience, University of Melbourne.

IRAP (insulin-regulated aminopeptidase) is a membrane bound metalloprotease, which is colocalised with the insulin-regulated glucose transporter GLUT4 in hippocampal and cortical neurons. Substrates of IRAP include vasopressin and oxytocin, the actions of which are important in several memory domains. Central infusion of IRAP inhibitors attenuates memory deficits in several rodent models, and we propose that IRAP represents a novel target for the development of cognition-enhancing drugs. We sought to identify the role of IRAP in neurons of the forebrain using the IRAP lox x CamKII $\alpha$ -Cre mouse. Animals carrying both the floxed IRAP gene and the CamKII $\alpha$  directed Cre recombinase have a forebrain-neuron specific deletion of IRAP, as confirmed by western blot and autoradiography. Male forebrain-neuron IRAP KO mice and their WT littermates were subjected to behavioural assessment at 3, 9 and 15 months. There was a statistically significant interaction between arm preference and genotype for 3-month old animals in the Y-maze ( $p=0.02$ , RMANOVA,  $n=19-21$ ). WT animals had intact spatial memory, spending 46.6% of the trial in the novel arm. However, KO mice did not perform above chance levels. For object recognition at the same age there was a statistically significant interaction between trial and genotype for the preference index ( $p=0.049$ , RMANOVA,  $n=13-16$ ). WT animals indicated a preference for the novel object (19.2% more exploration) whilst KO mice had no preference. Western blot analysis of cortical and hippocampal membrane homogenates indicated altered expression of GLUT4. Since GLUT4 is required for facilitated glucose uptake during high metabolic demand, the altered GLUT4 expression may explain the poor learning demonstrated by the forebrain-neuron IRAP KO mice.

## POS-TUE-202

**INSULIN-LIKE PEPTIDE 3 (INSL3) IN MOUSE BRAIN: SOMATIC NEURONAL EXPRESSION AND EFFECTS OF CENTRAL ADMINISTRATION ON COMPLEX BEHAVIOUR**

Karunaratne N.S., Sang Q., Sedaghat K., Lin F., Wade J.D. and Gundlach A.L.

Florey Neuroscience Institutes, The University of Melbourne, Victoria 3010, Australia.

Insulin-like peptide-3 (INSL3) is highly expressed by testicular Leydig cells and is present in the circulation of adult male rodents and humans, but neuronal expression has not been identified. Leucine-rich repeat containing G-protein coupled receptor 8 (LGR8), the native receptor for INSL3, is associated with excitatory pathways in mouse brain - the cortico-striatal and habenulo-interpeduncular systems, and in amygdala, midline thalamus, hypothalamus and brainstem, but its role is unknown. Therefore, these studies explored the possible presence of INSL3 expression in mouse brain and the effect of central LGR8 activation on mouse behaviour. Initial studies identified evidence of low levels of INSL3 mRNA in subcortical brain and the presence of Golgi-associated INSL3 immunoreactivity in soma of neurons in red nucleus and other areas. Groups of C57BL/6J mice ( $n=8-13$ ) received 4- $\mu$ l icv infusions of aCSF or INSL3 (80-320 pmol) and were assessed in the automated locomotor cell, and the novel-object exploration (NOE), social interaction, and anxiety/stress tests. INSL3 (80-160 pmol) had no effect on locomotor activity over 60 min, but 320 pmol induced hypoactivity 45-60 min post-injection ( $p < 0.05$ ). Exploration of inanimate objects in the NOE or other mice in a sociability/social novelty test was not altered by INSL3, but inter-male aggression was elevated after 160 pmol INSL3 ( $p < 0.001$ ). Multiple tests revealed evidence for increased anxiety-like behaviour after INSL3, both in a social and environmental context - intruder-recognition test ( $p < 0.05$ ), elevated plus maze ( $p < 0.05$ ) and light/dark box ( $p < 0.05$ ). The data suggest a role for INSL3/LGR8 signalling in sensorimotor and emotional behaviors in mice.

## POS-TUE-203

**CHARACTERIZATION OF A NOVEL COVALENTLY CROSS-LINKED A $\beta$  PEPTIDE DIMER AND ITS ROLE IN ALZHEIMER'S DISEASE**Ciccotosto G.D.<sup>1,2,3</sup>, Kok W.M.<sup>1,2,4</sup>, Naylor R.<sup>1,2</sup>, Tew D.<sup>1,2,3</sup>, Hutton C.A.<sup>2,4</sup>, Lal D.<sup>3</sup>, Bowser D.<sup>3</sup>, Masters C.L.<sup>3</sup>, Cappai R.<sup>1,2</sup> and Barnham K.J.<sup>1,2,3</sup><sup>1</sup>Department of Pathology. <sup>2</sup>Bio21 Molecular Science and Biotechnology Institute. <sup>3</sup>Mental Health Institute of Victoria. <sup>4</sup>School of Chemistry, The University of Melbourne, Parkville, VIC 3010, Australia.

Alzheimer's disease (AD) is the most common form of dementia and is characterized by progressive memory loss, confusion, and cognitive deficits. While the major cause in AD is unknown, it is generally accepted that the beta amyloid peptide (A $\beta$ ) is toxic and we are confident that the toxic species are a low molecular weight soluble oligomeric species of A $\beta$  that is responsible for neuronal dysfunction and synaptic loss. Furthermore, we propose that the formation of the toxic oligomeric species occurs via dityrosine crosslinking of the A $\beta$  peptide (dY-A $\beta$ ), the process which is brought about by an oxidative modification reaction. It is a fundamental question in understanding A $\beta$  neurotoxicity to test a bona fide dimeric A $\beta$  with no monomer present. The complexity of synthesizing dityrosine-containing dimeric A $\beta$  peptides has hampered research to directly test its toxic effects. The task of synthesizing an 84 amino acid peptide is a very difficult process and requires novel chemistry paradigms and experience in handling the A $\beta$  peptide. We have developed novel methods for successfully synthesizing the dY-A $\beta$  dimer peptides containing up to 84 residues with high yields of pure peptides. To date, we have successfully synthesized and purified a number of A $\beta$  monomers and respective dY-A $\beta$  dimers and control DAP-A $\beta$  dimers (this is the control dimer peptide using the 2,6-diaminopimelic acid in place of dityrosine during the synthesis of the A $\beta$  dimer). During the past five years, we have successfully investigated a number of mutant and modified A $\beta$  peptides using a combination of neurotoxicity, synaptotoxicity, aggregation, biophysical, and biochemical assays in order to decipher the mechanisms of A $\beta$  neurotoxicity. We now plan to subject the novel dY-A $\beta$  peptides to a number of these well established assays in our laboratories and we will present some of the preliminary findings that we have collated so far.

## POS-TUE-204

**STUDIES ON THE MECHANISM OF HEPARIN-INDUCED STIMULATION OF PROBACE1**Klaver D.W.<sup>1,2</sup>, Wilce M.<sup>1</sup>, Beckman M.<sup>1,3</sup>, Freeman C.<sup>4</sup>, Juliano J.-P.<sup>1</sup>, Parish C.<sup>4</sup>, Gasperini R.<sup>2</sup>, Foa L.<sup>2</sup>, Aguilar M.-I.<sup>1</sup> and Small D.H.<sup>2,1</sup><sup>1</sup>Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia. <sup>2</sup>Menzies Research Institute, University of Tasmania, Hobart, Tasmania 7000, Australia. <sup>3</sup>Life Sciences, Södertörns University, SE-141 89, Huddinge, Sweden. <sup>4</sup>Division of Immunology and Genetics, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.

The  $\beta$ -site APP cleaving enzyme (BACE1) is responsible for the first step in production of the  $\beta$ -amyloid protein (A $\beta$ ). BACE1 is synthesized as a partially active zymogen (proBACE1). We previously showed that heparin can increase the enzyme activity of proBACE1. In this study, the structural requirements and mechanism for the heparin-induced activation were examined. The effect of heparin on proBACE1 was influenced by the degree of sulfation ( $p < 0.001$ ) and carboxylation ( $p < 0.001$ ), as well as by the length of the sugar ( $p < 0.005$ ). Although low molecular weight heparin fragments did not strongly stimulate proBACE1, they inhibited heparin-induced activation of the enzyme ( $p < 0.001$ ). The zymogen structure was modeled using a known X-ray structure of the protease domain. The modeled structure suggested that a heparin-binding domain may reside near the prodomain, and that movement of loop region (residues 46 – 65) lying adjacent to the prodomain may be needed to accommodate heparin binding. This loop domain lies adjacent to the active site and may block access to the active site. The movement of this loop region upon heparin binding could expose the active site region to allow for increased substrate binding. The results suggest a model in which conformational changes close to the prodomain may be involved in the mechanism of heparin-induced activation.



## POS-TUE-205

## CELLULAR MODELS TO INVESTIGATE THE EFFECT OF OXIDATIVE STRESS ON BACE1

Tan J.<sup>1</sup>, Li Q.-X.<sup>1,2</sup> and Evin G.<sup>1,2</sup><sup>1</sup>Department of Pathology, University of Melbourne, Parkville 3010.<sup>2</sup>Mental Health Research Institute, Parkville 3052.

**Background and aim:** Alzheimer's disease (AD) is associated with plaques consisting of A $\beta$  peptides. BACE1 ( $\beta$ -site APP cleaving enzyme 1) initiates A $\beta$  generation in neurons by cleaving the amyloid precursor protein (APP). BACE1 is elevated in AD brain cortex, and studies have shown that it is elevated in cells during oxidative stress (OS); however how endogenous BACE1 might be accumulated within cells remains unclear. The aim is to study BACE1 in cell culture in an OS condition. **Methods:** SH-SY5Y cells were cultured to 70% confluence and treated with 0, 100, 200 and 400  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 3h or with 1mM buthionine sulfoximine (BSO) for 24h. Protein carbonyl detection was used to measure OS in H<sub>2</sub>O<sub>2</sub>-treated cells, whereas OS by BSO treatment was measured using 2',7'-Dichlorofluorescein (DCFH). Cell lysate was separated by SDS-PAGE and analysed by Western blotting. Statistical analyses were performed with the SPSS software (significance when  $p < 0.05$ ). **Results:** H<sub>2</sub>O<sub>2</sub> treatment resulted in a dose-dependent increase in protein carbonyl levels ( $n=6$ ), however no statistical significance was obtained. Accordingly, the increase in BACE1 levels ( $n=12$ ) were not significant. BSO was able to significantly induce OS ( $n=6$ ), but with no change in BACE1 levels ( $n=6$ ). **Conclusion:** Inducing OS in SH-SY5Y cells did not increase BACE1 levels. Studying of BACE1 in the context of OS may be better carried out in differentiated neurons. We are now investigating effect of OS on BACE1 in mouse primary cortical neurons.

## POS-TUE-207

## INVESTIGATION OF MATRIX METALLOPROTEINASE AS A BLOOD BIOMARKER FOR ALZHEIMER'S DISEASE

Lim N.<sup>1</sup>, Soon C.<sup>1</sup>, Laughton K.<sup>1</sup>, Villemagne V.<sup>1,2</sup>, Rowe C.<sup>1,2</sup>, Masters C.<sup>1</sup>, Evin G.<sup>1</sup> and Li Q.-X.<sup>1</sup><sup>1</sup>Dept of Pathology, University of Melbourne. <sup>2</sup>Dept of Nuclear Medicine, Austin Health, Australia.

Pathological changes in the Alzheimer's disease (AD) brain include the aggregation of A-beta and Tau proteins (in the form of plaques and tangles respectively), as well as neuronal death and synaptic loss. Matrix metalloproteinase -9 (MMP-9) and MMP-2 are able to degrade A-beta and their expression is increased in astrocytes near amyloid plaques. Similar changes may be reflected in plasma. We studied MMP-9 and MMP-2 activity in a well characterised plasma cohort consisting of healthy controls (HC,  $n=26$ ), Mild cognitive impairment (MCI,  $n=26$ ), AD ( $n=26$ ), Dementia with Lewy Bodies (DLB,  $n=12$ ) and Frontotemporal Lobar Degeneration (FTLD,  $n=9$ ) groups. Using zymography, MMP-9 and MMP-2 activity were detectable in all of the plasma samples. A significant decrease in MMP-2 activity was found in the AD group compared to the HC ( $p < 0.05$ ), though no significance was found for MMP-9 activity between the groups. Thus, in AD patients, a decrease in MMP-2 activity in plasma might indicate an impairment in A-beta degradation, which could contribute to the pathogenesis of AD. This study shows promise for MMP-2 to be used as a biomarker in plasma, which could aid in the early diagnosis for AD.

## POS-TUE-206

## SCREENING OF SPHINGOLIPID BIOSYNTHESIS INHIBITORS AS AMYLOID-BETA MODULATORS

Li H.<sup>1</sup>, Kim W.S.<sup>1,2</sup>, Hill A.F.<sup>3</sup>, Evin G.<sup>3</sup> and Garner B.<sup>1,2</sup><sup>1</sup>Prince of Wales Medical Research Institute, Randwick, NSW 2031,Australia. <sup>2</sup>School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney NSW 2052, Australia. <sup>3</sup>Department of Pathology University of Melbourne, VIC 3010, Australia.

Alzheimer's disease (AD) is characterized by cerebral amyloid deposits composed of the amyloid-beta peptide (A $\beta$ ) which is released by proteolytic processing of A $\beta$  precursor protein (APP). Amyloidogenic processing of APP occurs within cell membranes in lipid raft microdomains that are enriched with glycosphingolipids (GSLs), sphingomyelin (SM) and cholesterol. It is established that inhibition of cholesterol biosynthesis suppresses A $\beta$  production, whether modulation of cellular sphingolipid synthesis inhibitors on APP processing. Based on western blot analysis of secreted A $\beta$  and soluble APP alpha (sAPP $\alpha$ ), we demonstrate that a panel of inhibitors of glucosylceramide synthase: PDMP (D,L-threo-ethylenedioxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol), PPMP (D,L-threo-1-Phenyl-2-hexadecanoylamino-3-morpholino-propanol), EtDO-P4 (D,L-threo-ethylenedioxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol) and NB-DNJ (N-Butyl-deoxynojirimycin), reduced extracellular A $\beta$  concentrations with approximate IC<sub>50</sub> values of 0.020, 0.005, 0.001, 0.100 mM, respectively. In contrast to the well-characterized gamma-secretase inhibitor, DAPT (N-(3,5-Difluorophenyl)acetyl-L-alanyl-2-phenyl glycine-1,1-dimethylethyl ester, used at a concentration of 0.1  $\mu$ M), GSL inhibitor compounds did not significantly increase extracellular levels of sAPP $\alpha$  or intracellular levels of the APP C-terminal beta (CTF $\beta$ ) fragment. We also found that there was a significant reduction of A $\beta$  secretion after cells were treated with myriocin (2-amino-3,4-dihydroxy-2-(hydroxymethyl)-14-oxo-6-eicosenoic acid), a specific serine palmitoyl transferase inhibitor which reduces cellular GSLs and SM, or with D609 (tricyclodecan-9-yl-xanthogenate), or a SM synthase inhibitor (IC<sub>50</sub> values of 0.100, 0.300 mM, respectively). These results indicate the potential to develop anti-amyloidogenic compounds via targeting sphingolipid biosynthesis.

## POS-TUE-208

## THE UNIQUE C-TERMINAL REGION OF SECRETED AMYLOID PRECURSOR PROTEIN ALPHA IS CRUCIAL FOR NEUROPROTECTION

Mukadam A., Bourne K. and Tate W.

Department of Biochemistry, University of Otago, Dunedin, New Zealand.

Alzheimer's disease (AD) is characterised by increased concentration and aggregation of the toxic 'sticky peptide' amyloid beta (A $\beta$ ), and reduced concentrations of the neuroprotective protein secreted amyloid precursor protein alpha (sAPP $\alpha$ ). In the healthy brain the pathways for A $\beta$  and sAPP $\alpha$  production are balanced but in AD this equilibrium is disrupted. sAPP $\alpha$  differs from sAPP $\beta$ , produced in the A $\beta$  pathway, by having its C-terminus longer by 16 amino acids, and yet it is up to 100-fold more potent than sAPP $\beta$  in conferring protective effects on neurons. The exclusive C-terminal sequence of sAPP $\alpha$  is strongly hydrophilic (11/16 residues) and has a number of sites that might be functionally important for neuroprotection, for example the consecutive histidines that may form a metal-binding site, a basic charged terminal amino acid, and a postulated heparin-binding domain (VHHQK). Stable cell lines that expressed substitution and deletion variants of sAPP $\alpha$  at potentially important sites were generated, and the recombinant proteins purified from the cultured cell media using a one-step purification. They were tested for their ability to protect human neuroblastoma cells (SH-SY5Y) against hypoglycaemic insult, compared with wild-type sAPP $\alpha$ . sAPP $\alpha$  was able to protect the cells from the adverse effects of hypoglycaemia in a number of protocols, while the variants had different activities ranging from 30% less potent to being significantly toxic to the cells ( $n \geq 6$ ,  $p < 0.05$ ). A peptide corresponding to the exclusive C-terminus of sAPP $\alpha$  mimicked the protection offered by sAPP $\alpha$ , but protection was independent of sequence order, highlighting the importance of the hydrophilic character of this region ( $n=3$ ). This study confirms that the C-terminal region of sAPP $\alpha$  has a role in neuroprotection.

## POS-TUE-209

**EFFECT OF DIRECT CONTACT WITH A $\beta$  ON NEURITE OUTGROWTH IN AN IN VITRO MODEL OF DYSTROPHIC NEURITES**

**Percy N.L.**, Gasperini R., Foa L. and Small D.H.  
Menzies Research Institute, University of Tasmania, Hobart, 7001, Tasmania, Australia.

Alzheimer's disease (AD) is a progressive neurological disorder, and is the most common form of dementia in the elderly. One of the key pathological features in AD is the presence of dystrophic neurites surrounding amyloid plaques, which is thought to contribute to the cognitive decline in AD. In this study, an in vitro assay was created to examine the effect of direct contact of hippocampal neurons with a substrate-bound amyloid beta (A $\beta$ ) deposit. Evidence strongly suggests that A $\beta$  is the main neurotoxic agent underlying AD. Hippocampal neurons were seeded on and around A $\beta$  deposits and morphological features were compared using immunocytochemical staining techniques between neurons that were in contact with and neurons that were not in contact with the A $\beta$  deposit. This study showed that contact with A $\beta$ 40 and A $\beta$ 42 induced the collapse of growth cones ( $P < 0.0001$  for both A $\beta$ 40 and A $\beta$ 42) and increased pathogenic tau species phosphorylated at ser202/thr205 ( $P < 0.0001$  for both A $\beta$ 40 and A $\beta$ 42). Neuronal contact with A $\beta$ 42, but not A $\beta$ 40, increased the level of the neurodegenerative marker, ubiquitin ( $P < 0.0001$ ). A calcium imaging technique was also used to examine the effect of A $\beta$  on glutamate activity, and revealed that neurons grown in contact with substrate-bound A $\beta$  deposits exhibited an altered sensitivity to glutamate. These neurodegenerative changes are similar to those reported in dystrophic neurites observed in vivo and in vitro. Therefore, the A $\beta$ -contact assay was found to be a robust model which may be useful for studying the mechanisms of neuritic dystrophy in vitro.

## POS-TUE-210

**CALCIUM OSCILLATIONS IN GLIAL PRECURSORS INDUCED BY AMYLOID  $\beta$** 

**Vincent A.J.**, Gasperini R., Foa L. and Small D.H.  
Menzies Research Institute, University of Tasmania, Hobart, Tasmania.

Alzheimer's disease is caused by the amyloidogenic peptide, A $\beta$ , which causes dysregulation of calcium in neurons and astrocytes. Excitotoxicity associated with disrupted calcium signalling is thought to underlie the symptoms and pathology of Alzheimer's disease. However, the mechanism of A $\beta$ -induced calcium influx in brain cells is unknown. Recent work in our lab has shown that an amyloidogenic form of transthyretin, which causes familial amyloidotic polyneuropathy, triggers calcium influx into dorsal root ganglion cells via a transient receptor potential (TRP) channel. We therefore asked whether A $\beta$  acts in a similar manner to induce calcium dysregulation in CNS-derived cells. We grew cultures of dissociated cortical cells isolated from newborn mice for 1 week in vitro, and then treated the cultures with 1  $\mu$ M oligomeric A $\beta_{1-42}$  for 2-6h. Cells were loaded with 2  $\mu$ M Fluo-4 AM and calcium fluorescence images were captured on a Zeiss LSM 510 confocal microscope. Nearly twice as many cells in A $\beta_{1-42}$ -treated cultures displayed dynamic calcium activity compared to vehicle-treated control cultures (155% of control,  $n = 8$  cultures). A $\beta_{1-42}$  induced a shift from a spontaneous pattern of calcium activity to a regular spike pattern (55% of active cells vs 33% in vehicle-treated cultures). Immunostaining of imaged cells revealed that this was not attributable to MAP2+ neurons or GFAP+ astrocytes, and that significant populations of glial precursors were present in the cultures. Live staining suggests that the A $\beta$ -responsive cells may be NG2 cells. In conclusion, A $\beta_{1-42}$  induced an increase in the number of cells in cortical cultures with active calcium signalling, and promoted a pattern of regular spike activity. These active cells may be glial precursors, possibly NG2 cells.

## POS-TUE-211

**POST-SYNAPTIC SCAFFOLD PROTEIN LOSS CORRELATES WITH ALZHEIMER'S DISEASE PATHOLOGY**

**Proctor D.T.**<sup>1</sup>, Coulson E.J.<sup>2</sup> and Dodd P.R.<sup>1</sup>  
<sup>1</sup>School of Chemistry and Molecular Biosciences. <sup>2</sup>Queensland Brain Institute, University of Queensland.

Cognitive decline in Alzheimer's disease (AD) conforms strongly to region-specific synapse loss. In brain areas affected by AD, a concurrent decline in NMDA receptors has been reported. Glutamate-evoked excitotoxicity mediated through over-stimulation of NMDA receptors may contribute to synaptic degeneration in AD. NMDA receptor expression and activity at the synapse is highly regulated through binding to the post-synaptic scaffold proteins PSD-93, PSD-95, SAP-97 and SAP-102. Altered scaffolding could underlie NMDA receptor subunit loss in AD. Using absolute quantification Real-Time PCR, we detected no significant differences in expression of scaffolding protein mRNA transcripts between AD cases ( $n = 13$ ) and controls ( $n = 12$ ), in the pathologically affected hippocampus (Hip) and inferior temporal cortex (ITC). In contrast, the pre-synaptic protein synaptophysin was markedly less abundant in the ITC on post-hoc testing ( $P < 0.05$ ), consistent with previous reports. No differences in the expression of any of these transcripts were found in the occipital cortex (OC), a region spared from marked cell loss, between AD cases and controls. Proteins were precisely quantified against recombinant truncated protein standards. A significant decline in PSD-95 and SAP-102 protein expression was observed in the ITC on post-hoc testing ( $P < 0.05$ ), between AD cases ( $n = 15$ ) and controls ( $n = 15$ ) and for SAP-102 in the OC ( $P < 0.05$ ). Both transcript and protein levels declined significantly with the progression from absence of AD neuropathology to moderate stages ( $P < 0.001$ ). PSD-93, PSD-95 SAP-97 and SAP-102 immunohistochemical staining in Hip, ITC and OC sections, revealed differences between AD ( $n = 6$ ) and controls ( $n = 6$ ). Our data suggest a possible mechanism for reduction in NMDA receptor subunit expression in AD ITC. This research provides further understanding of the excitotoxic pathology of AD at the molecular level.

## POS-TUE-212

**NEUROPATHOLOGY OF SENILE PLAQUES AND EXPERIMENTAL HAEMORRHAGIC LESIONS: TEST OF THE MICROVASCULAR HYPOTHESIS OF PLAQUE FORMATION IN HUMAN BRAIN**

**Purushothuman S.**<sup>1,2</sup>, Marotte L.<sup>2</sup> and Stone J.<sup>1</sup>  
<sup>1</sup>Discipline of Physiology, Sydney Medical School and Bosch Institute, University of Sydney, NSW 2006, Australia. <sup>2</sup>School of Biology, Australian National University, Canberra ACT 0200, Australia.

A key unresolved issue in understanding age-related dementia (ARD, Alzheimer's disease) is the cause of formation of the senile plaques prominent in the ARD brain. This study tests the hypothesis that plaques form at sites of capillary haemorrhage. Small surgical lesions were made in neo- and hippocampal cortex of young adult rat brain ( $n = 20$ ), and the resulting pathology was examined after 1, 3, 7, 14, 19 and 30d, for haemorrhage (the Perl reaction), cell death (the TUNEL technique) and, using immunohistochemistry, for gliosis, neuronal survival, synaptic density, and the levels and location of amyloid precursor protein (APP) and A $\beta$ . Neocortex from the superior parietal and superior temporal lobes from ARD cases ( $n = 4$ ) was also examined, with the same techniques. Evidence of haemorrhage was found both at experimental lesions and in human plaques; neurones and synapses were depleted at both; micro- and macroglia (astrocytes) invaded both; small, autofluorescent extracellular deposits formed at both. Dying cells were detected only at the experimental sites within a few days of lesion. A $\beta$  was prominent extracellularly at the core of human plaques, in the human plaques and was expressed intracellularly, in neurones and neuroglia around the experimental lesion. These two differences between the experimental lesion sites and human plaques may reflect the relative maturity of plaques, which were observed in the post-mortem brain, as long as 7 years after diagnosis, whereas the experimental lesions were  $< 1$  month old. The similarities give support to the idea that plaques form at the sites of intracerebral haemorrhage and that ARD may, therefore, be a vascular dementia.

## POS-TUE-213

## DECREASES IN RECEPTOR-ASSOCIATED PROTEIN IN ALZHEIMER'S DISEASE HUMAN BRAIN TISSUE CORRELATE WITH INCREASED PLAQUE LOAD

Shepherd C.E.<sup>1,2</sup>, Hill M.<sup>1</sup>, Small D.<sup>3</sup> and Halliday G.M.<sup>1,2</sup><sup>1</sup>Prince of Wales Medical Research Institute, Sydney. <sup>2</sup>University of New South Wales, Sydney. <sup>3</sup>Menzies Research Institute, University of Tasmania.

Beta amyloid (A $\beta$ ) accumulation is a critical event underlying the pathogenesis of Alzheimer's disease (AD). Previous studies have shown that the receptor-associated protein (RAP) assists in maturation of a number of proteins involved in metabolism of the beta-amyloid precursor protein. In addition, RAP binds A $\beta$  and can inhibit its neurotoxic effects *in vitro*. However, no studies have assessed the association between RAP and A $\beta$  deposition in AD brain tissue. Brain tissue from 7 AD and 8 age-matched control cases with short postmortem delays was obtained from the Prince of Wales Medical Research Institute Brain Bank. Ten  $\mu$ m sections were cut from paraffin-embedded formalin-fixed tissue blocks of the hippocampus and peroxidase immunohistochemistry performed using anti-RAP (mouse monoclonal 7F1) and anti-A $\beta$  (IE8) with cresyl violet counterstaining. All cases were staged for the severity of A $\beta$  plaque deposition according to CERAD criteria. Analysis revealed that most RAP immunoreactivity was neuronal and intracellular. RAP-immunoreactive and immunonegative CA1 neurons were counted in standardised samples (11x11 eye piece grid at 200x magnification, 5% rater agreement) of the hippocampus and the percentage of RAP-immunoreactive neurons calculated. Analysis revealed  $89 \pm 3\%$  ( $\pm$  SEM) of control CA1 neurons contained RAP immunoreactivity whereas only  $38 \pm 11\%$  of AD CA1 neurons were RAP-immunopositive (t-test  $p = 0.003$ ). The numbers of RAP-immunoreactive neurons negatively correlated with the severity of A $\beta$  plaque pathology (Spearman correlation =  $-0.8$ ,  $p < .0001$ ). These data demonstrate significant decreases in neuronal RAP in association with A $\beta$  deposition in AD brain, supporting the concept that RAP is protective against A $\beta$  toxicity and aggregation.

## POS-TUE-215

## TAU DEPOSITION CORRELATES WITH INFLAMMATION IN ALZHEIMER'S DISEASE

Yeung P.K., Halliday G.M. and Shepherd C.E.  
University of New South Wales and the Prince of Wales Medical Research Institute, Sydney. AUSTRALIA.

Monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) are consistently upregulated in Alzheimer's disease (AD) brain and thought to play a role in disease pathogenesis. The major stimulator/s for this inflammatory response remain unclear. The aim of this study was to determine which major AD pathology (A $\beta$  plaque, hyperphosphorylated tau, or neuronal loss) most closely associates with MCP-1 and IL-6 containing cells. Brain tissue samples from the inferior temporal cortex of 8 AD, 6 cases with mild cognitive impairment (MCI) and controls with either A $\beta$  deposition, tau deposition, or no neuropathology ( $n = 22$ ) were obtained from the Prince of Wales Medical Research Institute Brain Bank. Ten  $\mu$ m sections were stained (peroxidase as well as fluorescence double-labelling) using antibodies against MCP-1 (anti-human MCP-1), IL-6 (anti-human IL-6) and tau (AT8) counterstained with cresyl violet for cellular quantitation. Neuronal density was determined in five counting frames in cortical layer III, and the proportion of immunoreactive neurons determined. Significant neuronal loss was found in AD ( $154 \pm 16/\text{mm}^2$ ) and MCI ( $140 \pm 6/\text{mm}^2$ ) compared to controls ( $254 \pm 14/\text{mm}^2$ , Kruskal Wallis, posthoc  $p \leq 0.05$ ). Immunoreactive glia (semi-quantitatively scored 0-3), cored and non-cored plaques (density and areal fraction) were also assessed. AD cases demonstrated a significant increase in MCP-1- and IL-6-immunoreactive glia compared with controls (average 200% increase, Kruskal Wallis, posthoc  $p \leq 0.05$ ). Stepwise multiple regression analyses ( $p \leq 0.05$ ) revealed that increasing MCP-1- and IL-6-immunoreactive glia most strongly and consistently correlated with increasing tau. Double labelling demonstrated a relationship between glial activation and neuritic pathologies. These results suggest that tau neuritic changes underlie the increased expression of MCP-1 and IL-6 immunoreactive glia in AD brain tissue.

## POS-TUE-214

## CHANGE IN GLUTATHIONE EXPORT PARAMETERS BY ACTIVATED ASTROCYTES - A NOVEL TARGET FOR ALZHEIMER'S DISEASE?

Steele M.L., Fuller S. and Muench G.

Department of Pharmacology, School of Medicine, University of Western Sydney.

**Purpose:** Astrocytes possess numerous neurosupportive functions, including the synthesis of glutathione (GSH). In pathological conditions, astrocytes are able to switch from metabolic support cells, to immunologic cells, capable of producing free radicals and cytokines, which can further exacerbate pathological conditions, including neurodegenerative disorders such as Alzheimer's disease (AD). Since AD is characterized by astro- and microglia activation, it is proposed that reduced astroglial GSH export could render neurons vulnerable to inflammatory and oxidative attack, possibly contributing to neurodegeneration. **Methods:** Thiols were measured by HPLC after pre-column derivatisation with 4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole. Cell viability was measured by the resazurin assay. **Results:** U373MG human astroglia were activated using various concentrations of the proinflammatory cytokines, IL-1 $\beta$  and TNF- $\alpha$ . It was shown that cytokine-activated astroglia initially (2-20hrs) increase the export of GSH in a cytokine concentration-dependent fashion (10 ng/ml IL-1 $\beta$ +TNF- $\alpha$  induced increased GSH export compared to 0-1 ng/ml;  $n=9$ ). After extended activation (30-72hrs) a decrease in GSH export was observed in response to 10ng/ml IL-1 $\beta$ +TNF- $\alpha$  compared to the untreated control and to astroglia treated with lower concentrations (0.01-1 ng/ml;  $n=9$ ). This decrease in astroglial GSH export is accompanied by a depletion of intracellular GSH. Furthermore, pre-treatment of astroglia with lipoic acid significantly increased GSH efflux, even in astroglia challenged with 10 ng/ml IL-1 $\beta$ +TNF- $\alpha$  ( $n=6$ ). Additionally, astroglia-conditioned media is able to protect SHSY5Y neurons against hydrogen peroxide toxicity in a manner that correlates with astroglial GSH export ( $n=6$ ). **Conclusion:** Therefore, low grade inflammation and lipoic acid increase astroglial GSH export and may be able to attenuate neuron death in AD and thus help slow the progression of the disease.

## POS-TUE-216

## GENERATION OF TAU-PROMOTER REPORTER CELLS FOR HIGH THROUGHPUT SCREENING

Liu X., Goetz J. and Ittner L.M.  
Brain and Mind Institute, University of Sydney.

The microtubule-associated protein tau is the major constituent of the paired helical filament, the main fibrous component of the neurofibrillary lesions of Alzheimer's disease. Its expression is neuronal specific and developmentally regulated. In this study, we generated two luciferase reporter vectors. The expression of luciferase is under the control of tau promoter (pTAU) and ubiquitin C promoter (pubc) respectively. Both constructs were stably expressed in SH-SY5Y and COS-7 cells by lentiviral gene transfer. Reporter cells were analysed by Western blot, immunocytochemistry and a luciferase activity assay. Using luciferin as substrate to determine luciferase activity is well-established, sensitive and effective, and therefore renders this system ideal for high throughput screening. The cell lines are currently used in a large array drug screening to identify compounds that can regulate tau expression. Candidate compounds will then be tested in transgenic mouse models to establish potential usage for treatment of diseases with tau pathology, such as Alzheimer's disease.



## POS-TUE-217

**QUINOLINIC ACID IN AIDS DEMENTIA COMPLEX WITH IMPLICATIONS FOR AN ALZHEIMER LIKE PATHOGENETIC COMPONENT**

Wu W.<sup>1</sup>, Stankovic R.<sup>2</sup>, Cullen K.M.<sup>3</sup>, Bell J.<sup>4</sup>, Brew B.J.<sup>5,6</sup> and Guillemain G.J.<sup>1,6</sup>

<sup>1</sup>Dept. of Pharmacology, University of New South Wales. <sup>2</sup>Dept of Pathology, University of Sydney. <sup>3</sup>Depts of Anatomy & Histology, University of Sydney. <sup>4</sup>Dept of Pathology, University of Edinburgh. <sup>5</sup>Dept of Neurology, St. Vincent's Hospital. <sup>6</sup>St Vincent's Centre for Applied Medical Research.

Human immunodeficiency virus-1 (HIV-1) infection of the central nervous system (CNS) can induce a dementing syndrome known as AIDS dementia complex (ADC). The mechanism remains poorly understood, evidence suggesting a relationship to Alzheimer disease (AD). The predominant pathogenesis of ADC is believed to involve activation of brain infiltrating macrophages and microglia. Quinolinic acid (QUIN) is an end product of tryptophan catabolism through the kynurenine pathway (KP) and is produced by activated macrophage/microglia. QUIN acts as an endogenous brain excitotoxin that leads to neuronal dysfunction. The present study investigated the frequency of QUIN, activated macrophage/microglia, and AD markers: amyloid beta (A $\beta$ ) and Tau in the hippocampus of 10 HIV-infected individuals. QUIN<sup>+</sup> staining was found in 70% (7/10) HIV<sup>+</sup> cases. Activated microglia/macrophages were 100% (10/10). Intra-neuronal A $\beta$  deposits were prevalent in all cases 100% (10/10); with four case 40% (4/10) having A $\beta$  plaque. Hyperphosphorylated Tau deposition was found in 30% (3/10) in all cases. Furthermore, using double staining, the infection of microglia/macrophage was demonstrated by detecting HIV p24 protein in microglia/macrophage in all cases. These microglia/macrophages were observed within and between QUIN<sup>+</sup> staining. HIV p24<sup>+</sup> staining was also shown with A $\beta$  plaque or Tau staining in some cases. Interestingly, QUIN<sup>+</sup> staining was co localised with either A $\beta$  plaque or Tau staining in these cases. We conclude that HIV-infected or immune-stimulated microglia/macrophage produce QUIN, and that there is a convergent pathogenetic mechanism with Alzheimer disease.

## POS-TUE-219

**N-CADHERIN,  $\beta$ -CATENIN AND GEPHYRIN LEVELS WITH PATHOLOGICAL SEVERITY IN ALZHEIMER DISEASE**

Tannenberg R.K.<sup>1</sup>, Worrall S.<sup>2</sup> and Dodd P.R.<sup>2</sup>

<sup>1</sup>School of Medicine, University of Queensland. <sup>2</sup>School of Chemistry and Molecular Biosciences, University of Queensland.

Alzheimer disease (AD) shows regional-specific loss of neurones and synapses. N-cadherin is a synaptic junction protein which bridges pre- and postsynaptic excitatory terminals via  $\beta$ -catenin attachment to the cytoskeleton. Gephyrin is an inhibitory postsynaptic scaffolding protein which associates with GABA<sub>A</sub> and glycine receptors. Brain tissue from pathologically confirmed AD and control cases was obtained at autopsy with informed written consent and frozen at -80°C in 0.32 M sucrose. Areas investigated included hippocampus and inferior temporal cortex, which are susceptible to AD pathology, and occipital cortex, which is relatively spared. Levels of N-cadherin,  $\beta$ -catenin, and gephyrin were measured by "in-gel" immunodetection on crude membrane preparations of frozen human brain tissue. Significantly higher levels of N-cadherin and  $\beta$ -catenin were observed in AD cases (n = 15) than in controls (n = 15), particularly in the hippocampus. Gephyrin levels were significantly reduced in AD cases compared with controls. Cases were scored according to pathological severity accounting for neuronal loss, tangle and plaque load, and gliosis. A score of 0 indicated no pathology; 1, mild or modest pathology; 2, moderate pathology; and 3, severe pathology, for each area studied. N-cadherin and  $\beta$ -catenin levels showed a significant, positive correlation with increasing pathological score. The levels of synaptophysin and GFAP were measured by indirect ELISA, to gauge the extent of synapse loss and reactive astrogliosis respectively. Synaptophysin levels were significantly lower, and GFAP levels were significantly higher in susceptible areas of AD cases than controls. GFAP levels correlated strongly and positively with increasing pathological severity, but synaptophysin correlated negatively and poorly with increasing pathological severity. These results could indicate a dysfunction of excitatory synapses in AD with differential pathology.

## POS-TUE-218

**MICROTUBULE-ASSOCIATED PROTEIN TAU PHOSPHORYLATED AT SPECIFIC RESIDUES IS RECRUITED TO ADF/COFILIN-ACTIN NEURITIC AGGREGATES: PATHOGENIC MECHANISMS IN EARLY ALZHEIMER'S**

Whiteman I.T.<sup>1,2</sup>, Minamide L.S.<sup>3</sup>, Bamburg J.R.<sup>3</sup> and Goldsby C.<sup>1</sup>

<sup>1</sup>Brain & Mind Research Institute. <sup>2</sup>Bosch Institute, University of Sydney, Australia. <sup>3</sup>Department of Biochemistry & Molecular Biology, Colorado State University USA.

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterised by three neuropathological hallmarks: neurofibrillary tangles, striated neuropil threads, both of which are comprised of phosphorylated microtubule-associated protein tau, and  $\beta$ -amyloid plaques. Almost always associated with tau and  $\beta$ -amyloid AD pathologies are aggregates of the actin-associated protein cofilin, which take on a characteristic rod-like structure similar to tau neuropil threads. While mechanisms underlying initiation of the disease remain poorly understood, increasing evidence suggests reduced cellular metabolism and mitochondrial function to be critical early events in the pathogenesis of sporadic AD. In recent work, we have demonstrated that mitochondrial inhibition in primary neuron culture triggers rapid activation of the actin-associated proteins cofilin and actin depolymerizing factor (ADF) which subsequently recruit and co-localize with phosphorylated tau. The resulting rod-shaped aggregates bear striking resemblance to the cofilin aggregates and tau neuropil threads observed in human AD brains. We are now interested in characterizing the phosphorylation patterns of this recruited tau, with emphasis on the Alzheimer-related phospho-epitopes. RESULT: Here we demonstrate that recruitment of tau to ADF/cofilin-actin aggregates is indeed dependent on its phosphorylation at specific residues. Interestingly, we observe a predominant recruitment of tau phosphorylated in the Microtubule Binding Domain (Ser262/Ser356), epitopes widely regarded as the first to be phosphorylated in AD. We propose that the formation of rod inclusions containing ADF/cofilin and phosphorylated tau most probably represent a very early event in AD neurodegeneration and as such, this work identifies a useful target for the development of AD therapeutics and treatment.

## POS-TUE-220

**CHARACTERISATION OF BRAIN DERIVED APOLIPOPROTEIN-E PEPTIDES USING IMMUNOPRECIPITATION AND MASS SPECTROMETRY**

Elliott D.A.<sup>1</sup>, Rafferty M.<sup>2</sup>, Halliday G.M.<sup>1</sup> and Garner B.<sup>1</sup>

<sup>1</sup>Prince of Wales Medical Research Institute, Randwick, NSW 2031, Australia. <sup>2</sup>School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia.

Apolipoprotein-E (apoE) is a polymorphic protein with an important role in neurobiology. There are three major isoforms of apoE (E2, E3, E4) and possession of the epsilon 4 allele is the greatest genetic risk factor for late onset Alzheimer's disease (AD). Previous work by our group has demonstrated that apoE undergoes proteolysis in the human brain and is present as a series of tris buffered saline (TBS) soluble peptides. ApoE proteolytic peptides were detected at significantly greater concentrations in apoE3 subjects compared to apoE4 subjects and this was independent of AD status. In the current study, immunoprecipitation was used to purify several apoE peptides from the brain homogenate of an apoE3 subject. The amino acid sequences of these peptides were deduced using mass spectrometric analysis. Currently, the two most abundant peptides, of approximately 25 and 26.5 kDa, have been characterised in detail with respect to the presence or absence of key functional regions of the apoE protein. The lipid-binding C-terminal domain was absent from the 25 kDa peptide, but present in the 26.5 kDa peptide, whereas the N-terminal domain was completely intact in the 25 kDa peptide and partially present in the 26.5 kDa peptide. Interestingly, the LDL-receptor binding region of the N-terminal domain was present in both peptides and the protease-sensitive inter-domain hinge region of apoE was intact only in the 26.5 kDa peptide. This data suggests that the major apoE fragments detected in the human brain are N-terminal peptides that contain the functionally important LDL receptor-binding region. The sequence analysis information will also be useful for identifying the enzymatic cleavage sites, and thus the responsible enzymes, for apoE proteolysis in the brain.

## POS-TUE-221

**COMPARTMENTALIZED EXCITOTOXICITY IN PRIMARY MOTOR NEURONS: A NOVEL MODEL OF ALS PATHOLOGY**

**Hosie K.A.**, King A.E., Vickers J.C. and Dickson T.C.  
Menzies Research Institute, University of Tasmania.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by distal axon degeneration prior to symptoms. Excitotoxicity has been implicated as a major factor in ALS pathogenesis. We have linked excitotoxicity in primary motor neurons to ALS-like distal axon degeneration. It remains unclear if toxicity is directed through the distal axon or somatodendritic compartment. To investigate the site of toxicity we have constructed an in vitro model utilising compartmentalized chambers. Growth of primary cortical neurons in these chambers leads to distinct somatodendritic and distal axon separation as evidenced by immunolabelling with MAP2 (dendritic marker) and tau (axonal marker). Cultures within the chambers (n=5) were focally exposed to glutamate (100µM, 24 hours). Somatodendritic treated neurons demonstrated degeneration of axons and dendrites, whilst axon treated neurons did not. However, evidence of distal axon synaptic maturity was absent in this paradigm. To further develop this model and investigate the role of the cell body/astrocyte and distal axon/muscle interactions in development of axonopathy, chambers were utilised such that motor neuron cell bodies were grown on astrocytes with the distal axon terminals on a substrate of primary skeletal muscle cells (from neonatal rats). This model mimicked many of the interactions of the lower motor neuron, including formation of rudimentary neuromuscular-synapses. Motor neuron growth on different substrates was determined, with significantly (p<0.05) diminished survival seen on muscle feeder layers. Mature (21DIV) cultures were subsequently exposed to chronic kainic acid (25µM, 24 hours), resulting in a significantly (p<0.05) increased loss of neurons co-cultured with astrocytes in comparison with the other substrates. These data suggests that non-neuronal cells can contribute to neuronal vulnerability to excitotoxicity. Further manipulations of this model may reveal skeletal muscle involvement.

## POS-TUE-223

**NEURONAL LOSS IN THE CINGULATE CORTEX IN FRONTOTEMPORAL DEMENTIA**

**Pok K.<sup>1</sup>**, Brooks D.<sup>2</sup>, Davies R.R.<sup>3</sup>, Hodges J.R.<sup>2</sup>, Xuereb J.H.<sup>4</sup>, Halliday G.H.<sup>2</sup> and Kril J.J.<sup>1</sup>

<sup>1</sup>Department of Pathology, University of Sydney. <sup>2</sup>Prince of Wales Medical Research Institute. <sup>3</sup>Department of Clinical Neurosciences, University of Cambridge. <sup>4</sup>Department of Pathology, University of Cambridge.

Frontotemporal dementia (FTD) has clinical variants that are characterised by behavioural (behavioural-variant FTD, bv-FTD) or language (semantic dementia, SD) impairments. The anterior cingulate cortex (ACC) is known to be affected early in bv-FTD, but regional vulnerability in other cingulate cortices and other FTD subtypes has not been evaluated. With approval from institutional ethics committees, paraffin-embedded samples were obtained from cases with cortical volume measurements from the POWMRI and Cambridge University Brain Banks. Samples from the ACC (Brodmann area 24) and posterior cingulate cortex (PCC; BA 23) were cut at 10µm and stained with cresyl violet and immunohistochemically for inclusions (AT8-tau and TDP-43). Non-biased quantitative techniques were used to estimate the neuronal number in bv-FTD (n=12), SD (n=6) and control (n=10) subjects. In the ACC, both groups showed an average 60% reduction in total neuron number compared with controls (p<0.01). In SD the PCC was similarly affected (p<0.01). However in bv-FTD the reduction failed to reach significance because of the wide variation in number between cases (16-61 million). This wide variation was also reflected in the volume measurements for these regions (2.2-5.9 mL). With respect to inclusion pathology, the ACC exhibited more inclusions than the PCC suggesting neuronal dysfunction in addition to neuronal loss. The greater damage to the PCC in SD may contribute to the different symptoms observed in SD compared with bv-FTD. The findings also reinforce the involvement of the ACC in FTD syndromes.

## POS-TUE-222

**HYPOTHALAMUS IN BEHAVIOURAL-VARIANT FRONTOTEMPORAL DEMENTIA**

**Lam B.Y.K.<sup>1,2</sup>**, Gabery S.<sup>3</sup>, Murphy K.<sup>1</sup>, Petersen A.<sup>3</sup>, Hodges J.R.<sup>1,2</sup>, Halliday G.M.<sup>1,2</sup> and Piguet O.<sup>1,2</sup>

<sup>1</sup>Prince of Wales Medical Research Institute, Sydney, Australia. <sup>2</sup>The University of New South Wales, Sydney, Australia. <sup>3</sup>Translational Neuroendocrine Unit, Lund University, Lund, Sweden.

Behavioural-variant frontotemporal dementia (bvFTD) is a progressive neurodegenerative brain disorder, characterised clinically by changes in behaviour (including feeding and sleeping disturbances) and cognition. The hypothalamus plays a critical role in homeostatic body functions such as feeding and sleeping, yet neuropathology in this region has not been assessed previously in bvFTD. We aimed to determine the severity of atrophy and cell loss in the hypothalamus in bvFTD, and the relations to feeding and sleeping behaviours. Two cohort studies were performed. Study 1 investigated early bvFTD cases (N=18) and matched controls (N=16) who had undergone structural MRI. Information on feeding and sleeping habits was collected from carer questionnaires. Hypothalamus volumes were traced manually on coronal images. Study 2 investigated postmortem bvFTD cases (N=12) and matched controls (N=6). Fixed coronal hypothalamic tissue blocks were serially sectioned and stained for Nissl substance and immunohistochemically for peptides regulating feeding and sleeping behaviours. Unbiased stereological estimates of hypothalamus volume and the number of neurons and glia were performed. Significant atrophy of the hypothalamus in bvFTD (posterior > anterior) was present in both studies. Behaviourally, patients with high sleep disturbance had significantly reduced anterior hypothalamus whilst patients with high feeding disturbance exhibited significantly reduced posterior hypothalamic volume. Neuronal loss, which was observed only in bvFTD cases with TDP-43, was predominant posteriorly and related to the degree of atrophy. Importantly, however, orexin neurons in the lateral hypothalamus that regulate feeding were spared. These data suggest that feeding disturbances in bvFTD indicates TDP-43 pathology and relates to posterior hypothalamic atrophy caused by neuronal loss.

## POS-TUE-224

**INCREASED IRON RELATED MR PHASE SIGNALS IN THE STRIATUM IN HUNTINGTON'S DISEASE: A NOVEL MR NEURODEGENERATIVE BIOMARKER**

Faggian N.<sup>1,2</sup>, Georgiou-Karistianis N.<sup>4</sup>, Chen Z.<sup>1</sup>, Bohanna I.<sup>1</sup>, Pia Carron S.<sup>4</sup>, Johnston L.<sup>1,3</sup>, Stout J.<sup>4</sup>, Churchyard A.<sup>4</sup> and **Egan G.<sup>1,2</sup>**

<sup>1</sup>Howard Florey Institute. <sup>2</sup>Centre for Neuroscience, University of Melbourne. <sup>3</sup>Department of Electrical and Electronic Engineering, University of Melbourne. <sup>4</sup>Centre for Developmental Psychiatry, Monash University.

**Introduction:** Our objective was to investigate novel in-vivo biomarkers of neurodegeneration in Huntington's disease (HD) for use in future clinical drug trials. MR phase signals are produced by magnetic susceptibility changes and can be used as an MR surrogate measure of iron concentration increases known to occur in HD. We have developed a novel method to investigate and quantify iron concentration in the basal ganglia of HD patients. **Patients and Methods:** Ten symptomatic HD patients (mean UHDRS 27.3) and ten controls were scanned using a T2\* imaging sequences on a 3T MRI. The T2\* scans were reconstructed using a complex image optimised reconstruction method to produce magnitude and phase images. For each phase image, the corpus callosum, caudate, globus pallidus and putamen were segmented manually. For voxels in each region the X84 outlier rejection rule was applied and the median phase signal was computed (Hz/Tesla). A univariate analysis of variance was computed to identify significant differences between groups. Partial correlations were computed between phase signals and relevant neurological scores, including: UHDRS, HADS, STROOP, SDMT, SCOP, FrSB and UPSIT. **Results** Compared to controls, there was a significant increase of the phase signal in HD patients in the globus pallidus (+0.20 Hz/Tesla, p=0.005) and putamen (+0.25 Hz/Tesla, p=0.01). Moreover, there was a significant (p < 0.005) positive correlation between the UHDRS score and the phase signal in the putamen. **Conclusion:** We have developed a novel approach to investigate changes of iron concentration in-vivo. This one of the first studies to quantify iron levels in patients with HD suggesting that MR phase signals, as a surrogate measure of iron concentration, may serve as a potential biomarker of neurodegeneration in HD. The finding of a significant relationship between UHDRS motor score and iron concentration give this finding clinical applicability.

## POS-TUE-225

## THE EFFECT OF SOCIAL DEFEAT ON TYROSINE HYDROXYLASE PHOSPHORYLATION IN RAT BRAIN AND ADRENALS

**Ong L.K.**, Bobrovskaya L., Walker F.R., Day T.A., Dickson P.W. and Dunkley P.R.  
School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia.

Tyrosine hydroxylase (TH) is regulated acutely by protein phosphorylation and chronically by protein synthesis. In these studies we aimed to investigate the phosphorylation of TH in rat brain and adrenals occurring within the first 24 hr in response to the social defeat stress. Intruders were exposed to social defeat (SD+) and then were sacrificed 10 min (n=6), or 24 hr (n=6), after the end point of the protocol. Sham intruders were not exposed to social defeat (SD-) but otherwise were treated identically to the intruders and were sacrificed 10 min (n=6), or 24 hr (n=6), after the end point of the protocol. Adrenals and brains were dissected and TH phosphorylation at serine residues 40, 31 and 19 (Ser40, Ser31 and Ser19) was analysed by western blotting. In the adrenals, pSer40 levels were significantly decreased at 24 hr (2 fold,  $p < 0.01$ ) but not at 10 min in SD+ animals compared to SD- animals. In the locus coeruleus and ventral tegmental area TH phosphorylation in SD+ animals was not significantly different from that in SD- animals at any time. In substantia nigra, pSer40 levels were significantly increased at 10 min (1.7 fold,  $p < 0.01$ ) and were significantly decreased at 24 hr (1.5 fold,  $p < 0.05$ ) in SD+ animals compared to SD- animals. We provide evidence for the first time that TH phosphorylation in rat adrenals and substantia nigra is modulated over time in response to social defeat and this may lead to changes in TH activity.

## POS-TUE-227

## LIPIDOMIC ASSESSMENT OF PARKINSON'S DISEASE BRAIN

**Cheng D.**<sup>1,2</sup>, McCann H.<sup>1,2</sup>, Halliday G.<sup>1,2</sup>, Nealon J.<sup>3</sup>, Mitchell T.<sup>4</sup> and Garner B.<sup>1,2</sup>

<sup>1</sup>Prince Of Wales Medical Research Institute, Randwick, NSW 2031, Australia. <sup>2</sup>School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia. <sup>3</sup>School of Chemistry. <sup>4</sup>School of Health Sciences, University of Wollongong, Wollongong, NSW, Australia.

Parkinson's disease (PD) is a neurodegenerative disorder affecting approximately 1% of the population aged 65 to 69 years, rising to approximately 3% of the population aged 80 years and older. Lewy bodies are neuropathological inclusions found in PD brain that are enriched in aggregated alpha-synuclein (Asyn) and lipids. Asyn is predicted to play a role in lipid homeostasis and Asyn null mice display a CNS lipid phenotype. **Aim:** To determine the lipid profile of anterior cingulate cortex, amygdala, and occipital cortex of human Parkinson's disease and age-matched control brains and correlate any detected changes with expression of Asyn protein. **Methods:** Pilot studies examined the occipital cortex using electrospray ionisation mass spectrometry (MS), high-performance liquid chromatography (HPLC) and western blotting (to detect the different aggregated forms of Asyn). **Results:** Several major sphingomyelin and ceramide species were accurately quantified (SEM < 12%, n = 4) by MS. In total, 23 molecular sphingomyelins and 6 molecular ceramides were identified, with species containing stearate (18:0) or nervonate (24:1) dominating both classes. Cholesterol, alpha-tocopherol and dolichol species were clearly detected by reversed-phase HPLC and a three-step fractionation method was established that can quantify soluble and insoluble fractions of Asyn protein. **Conclusion:** This research establishes reliable MS and HPLC methods that will be used to conduct a lipidomic assessment of the major sphingolipid and sterol species in Parkinson's disease and control brains.

## POS-TUE-226

## INVESTIGATION OF THE LOSS OF ASTROCYTES AS THE INITIATION OF PARKINSON'S DISEASE

**Tiong V.** and Allbutt H.N. Department of Physiology, University of Sydney, NSW 2006.

The cause of sporadic Parkinson's disease (PD) is unknown. There is no evidence of any myelin involvement; there has been no insult to neurons demonstrated that is capable of producing the pattern of degeneration observed and the consensus of the literature is that the activation of microglia is secondary to the neuronal degeneration associated with the condition. One possibility that could give rise to the pathologies associated with the condition is the loss of astrocytes. Astrocytes are the support cells of the brain and have been shown to be vital to the continued function and survival of neurons. It is hypothesized that a loss of astrocytes is the initiating trigger for the pathologies associated with PD. The present study examined the loss of astrocytes as a possible initiating trigger of the pathologies associated with PD by ablating astrocytes in the substantia nigra pars compacta (SNc; n=24). The number of tyrosine hydroxylase labelled neurons (marker for dopamine producing cells) and the metabolic activity of the nucleus using cytochrome oxidase was examined. This group of animals was compared to animals treated with 6-hydroxydopamine (positive control for the loss of dopamine; n=19), saline injected sham (n=16) and naive (n=11) group of rats. The effect was examined at 28 days and 56 days survival time. We found that loss of astrocytes did not appear to alter the number of dopamine producing cells however there was a significant decrease in the level of metabolic activity in the SNc ( $p < 0.05$ ). It was suggested that this change in metabolism may reflect early changes involved in the initiation of PD and that this may prove useful in the study of early pathologies.

## POS-TUE-228

DEVELOPMENT OF AN *IN VITRO* MODEL OF HUMAN DOPAMINERGIC NEURONS

**Zinger A.**<sup>1</sup>, Double K.<sup>2</sup> and Guillemin G.J.<sup>1,3</sup>

<sup>1</sup>UNSW, Dept of Pharmacology. <sup>2</sup>Prince of Wales Medical Research Institute. <sup>3</sup>St Vincent's Centre for Applied Medical Research.

Parkinson's disease (PD) is a progressive degenerative disorder resulting from a degeneration of dopaminergic neurons in *Substantia Nigra*. The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. The KP is activated in several neuroinflammatory diseases and is likely to be involved in PD pathogenesis. A prolonged activation of the KP leads to production and accumulation of the potent excitotoxin quinolinic acid (QUIN). We hypothesise that the KP in human dopaminergic neurons will lead to the production of neuroprotective KP metabolites and that these neurons will be very sensitive to QUIN toxicity as we previously found in cortical neurons. We aim to establish an *in vitro* model of human dopaminergic neurons to characterize the KP in Tyrosine Hydroxylase<sup>+</sup> (TH) neurons. We have differentiated human neuroblastoma SH-SY5Y (n=3) and SK-N-SH (n=3) cell lines using 3 different sets of treatments and then characterised them for neuronal and dopaminergic markers. Both cell lines treated with retinoic acid in with serum for 5 days followed by BDNF in serum free media for 5 days exhibited the morphology of dopaminergic neurons with fusiform and multipolar cell bodies and projecting dendrites. We will also assess expression of specific markers. This *in vitro* model will provide an important tool to study the involvement of QUIN in the death of TH<sup>+</sup> neurons and also the neuroprotective ability of KP inhibitors as potential therapeutic for PD.



## POS-TUE-229

## SUBSTANTIA NIGRA DOPAMINERGIC CELL RESPONSE TO ROTENONE INDUCED OXIDATIVE STRESS IN VIVO

Norazit A.<sup>1,2</sup>, Meedeniya A.C.B.<sup>1</sup>, Nguyen M.<sup>1</sup> and Mackay-Sim A.<sup>1</sup><sup>1</sup>National Centre for Adult Stem Cell Research, Griffith University, Brisbane, Queensland, Australia. <sup>2</sup>Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Rotenone, a high-affinity inhibitor of the mitochondrial complex 1 electron transport chain causes oxidative damage in dopaminergic neurons via production of reactive oxygen species. We examine oxidative damage related changes in the neuronal and glial cell population of the substantia nigra in response to focal rotenone challenge. Adult male Sprague-Dawley rats (n=3/group) were injected with 6, 3, 1, 0.5, and 0.25µg of rotenone focally into the substantia nigra or the medial forebrain bundle and sacrificed 14 days post infusion. Treatment was compared to a sham and 6-OHDA infusion. Brains were analysed using immunohistochemical techniques to identify dopaminergic neurons, their projections, glial cells, synapses, oxidative stress, and cell stress. Infusion of 6, 3, 1, 0.5, and 0.25µg of rotenone into the substantia nigra causes extensive damage and tissue necrosis and therefore limited in its relevance as an oxidative stress disease model. Infusion of 0.5µg of rotenone targeting the medial forebrain bundle caused a reduction in TH and synaptophysin density in the striatum (p<0.01), and a reduction in TH positive cells (p<0.01) and an increase in GFAP and IBA-1 positive cells (p<0.01) in the substantia nigra. Rotenone at 0.5µg also induced oxidative stress in dopaminergic neurons causing ongoing cell stress. Initial data in rats (n=18) infused with a low focal dose of rotenone produced a progressive reduction in striatal TH density over 60 days (p<0.01). These findings are supportive of a chronic model of neurodegeneration, where cellular interactions and pathways involved in degenerating dopaminergic neurons may be elucidated.

## POS-TUE-231

## WNT PROTEINS IN REMODELING AND REPAIR OF THE MIDBRAIN DOPAMINE PATHWAYS

Blakely B.D., Fernando C.V., Horne M.K., Thompson L.H. and Parish C.L. Florey Neuroscience Institutes, The University of Melbourne, Parkville, Victoria, Australia.

Repairing the injured or diseased brain will most likely rely on similar guidance cues as necessary for development. Recently we showed that Wnts play an important role in the development of midbrain dopamine (DA) pathways. These DA pathways are affected in a number of neurological disorders including Parkinson's disease (PD). Understanding regulators of adult DA axon remodeling may open new avenues to promote repair, in particular cell replacement therapy (CRT) for PD. The role of Wnts in adult remodeling was investigated using models established in our lab that illustrate the plasticity of the DA axons. Chronic haloperidol treatment (a DA receptor antagonist) results in a 40% increase in DA axonal branching and synapses in the target striatum. We chronically administered haloperidol to adult tyrosine hydroxylase-GFP mice (TH-GFP mice, in which all DA neurons are GFP+), isolated and dissociated the ventral midbrain and sorted (FACS) DA neurons (GFP+) from other VM cells (GFP-). Using qRT-PCR analysis DA axonal sprouting was confirmed in haloperidol mice by an upregulation of Gap43 and Synaptophysin compared to control mice. Interestingly, a number of Wnts and Wnt related receptors were also upregulated. A limitation of CRT for PD is the inadequacy of the grafted cells to reinnervate the striatal tissue. We therefore examined the ability of Wnts to promote axonal growth of grafted DA neurons in animal models of PD. Parkinsonian mice receiving fetal grafts as well as adjacent Wnt5a over-expressing grafts showed enhanced neuritegenesis and directional axon growth compared to fetal VM grafts + control cells. These findings suggest that Wnts are regulated in the remodeling brain and can be harnessed to promote repair.

## POS-TUE-230

## PARKINSON'S DISEASE PATIENTS CAN ADAPT TO PERTURBED VISUAL FEEDBACK WITH SUFFICIENT TRAINING

Leow L., Loftus A.M. and Hammond G.R. School of Psychology, University of Western Australia.

Parkinson's Disease (PD) patients appear to have difficulty adapting goal-directed movement to perturbed visual feedback in the visuomotor adaptation task paradigm. The mechanisms through which this occurs is unclear. **Purpose:** (1) To investigate if PD patients can achieve visuomotor adaptation to performance levels of controls given sufficient training (2) Evaluate the mechanisms through which PD patients achieve visuomotor adaptation. **Methods:** All participants (14 PD, 14 age-matched controls and 14 young controls) were trained to similar performance levels (movement direction within 3 degrees of the ideal trajectory) in a target-reaching task during practice. Visual feedback of movement trajectory was shown after movement completion. During the test phase, visual feedback of movement trajectory was rotated 30 degrees counter-clockwise. Participants adapted by reducing error in movement direction (directional error). Participants completed 4 blocks of 25 adaptation trials. One no-perturbation trial was interleaved at the end of each block to assess aftereffects. Adaptation rate was calculated by the rate at which directional error decreased (degrees/trial). **Results:** Across all blocks, adaptation rate was fastest in young controls (m = 3.40), followed by age-matched controls (m = 2.58) and PD patients (m = 0.86). Differences in adaptation rate between age-matched controls and PD patients reduced from 0.81 in Block 1 to 0.35 by Block 4. This indicates that PD patients adapt more slowly than controls, and may need more adaptation trials to achieve similar performance levels. Despite the absence of online visual feedback, PD patients may have used online kinesthetic feedback to correct movement error during adaptation. This is supported by the prevalence of submovements in PD movement trajectories, which were largely absent in controls. Submovements are believed to correct movement error online. **Conclusion:** Given sufficient exposure to perturbed visual feedback, PD patients can achieve visuomotor adaptation, however the mechanisms through which this is achieved may be different from controls.

## POS-TUE-232

## DOPAMINE RECEPTOR EXPRESSION CHANGES IN THE NUCLEUS ACCUMBENS OF RATS DISABLED BY PERIPHERAL NERVE INJURY

Austin P.J. and Keay K.A. School of Medical Sciences, University of Sydney, NSW, Australia, 2006.

**Purpose:** Chronic neuropathic pain is characterised by sensory changes, disability and altered affect. We have shown that following sciatic nerve constriction injury (CCI), 30% of rats develop *persistent disabilities* (altered social behaviour, sleep and endocrine function). Altered affect in chronic pain states may result from changes in the mesolimbic dopaminergic 'reward-aversion' circuitry of the nucleus accumbens (NAcc). The **aim** of these studies was to quantify changes in the NAcc by examining dopamine receptor mRNA and protein expression and the density of D2 receptor expressing neurons, in rats with *persistent disability* (n=6) and *no disability* (n=6) following CCI. **Methods:** Rats underwent sensory testing, as well as resident-intruder testing for 5 days prior to, and 6 days after CCI. Following testing, brains were processed for dopamine receptor expression using RT-PCR and Western blots, or D2 immunoreactivity (-IR) analysed stereologically. **Results:** There were decreases in mRNA expression of dopamine receptor sub-types in the NAcc of *persistent disability* animals [p>0.05 vs. *no disability*]; specifically D1 (contralateral) and D2 & D3 (ipsilateral). There was a significant decrease in D2 protein expression contralaterally in *no disability* animals [p>0.05 vs. *persistent*]. There were topographically specific decreases in the density of D2-IR neurons in the contralateral NAcc (2.5 mm to bregma) of *no disability* [p>0.05 vs. control] and in the ipsilateral NAcc (1.0 mm) of *persistent disability* animals [p>0.01 vs. control]. **Conclusion:** A subpopulation of rats, displaying sensory and affective disabilities, show highly lateralised neural (mal-)adaptations in reward-aversion circuitry of the NAcc after unilateral CCI. These changes appear to have major consequences on social behaviour, and may be involved in manifestation of disability and altered affect following nerve injury.

## POS-TUE-233

## PARKINSON'S DISEASE AND INSTABILITY WITHIN THE PARK2 LOCUS

Stephenson S.E.M.<sup>1,2</sup>, Feng Z.P.<sup>3</sup>, Taylor J.M.<sup>1</sup> and Lockhart P.J.<sup>1,2</sup><sup>1</sup>Bruce Lefroy Centre for Genetic Health, Murdoch Childrens Research Institute, Royal Children's Hospital, Australia. <sup>2</sup>Department of Paediatrics, University of Melbourne, Australia. <sup>3</sup>Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Australia.

Genomic alterations in PARK2 (*PRKN*) are the most common cause of familial early-onset autosomal-recessive Parkinson's disease (PD). *PRKN* shares a bidirectional promoter with *Parkin Co-Regulated Gene* (*PACRG*). The genes span an exaggerated genomic interval of 2 Mb as both genes encode super expanded introns, and dominate the 3rd most fragile site in the human genome - FRA6E. The significance of this genomic architecture has not been investigated. We hypothesise that the super expanded introns of these genes are a consequence of an ancestral rearrangement, and underlie the genetic instability within FRA6E. To investigate the genomic architecture of *PRKN* and *PACRG* we identified orthologues in model organisms that are experimentally relevant to human biology. Emphasis was placed on identifying the chromosomal location, genomic size and conservation of the bidirectional promoter. The genomic structure and bidirectional promoter of the *PRKN*-*PACRG* locus was conserved in mammals. In contrast, while both genes were located in the head-to-head orientation in birds the bidirectional locus was not maintained. Within the fish, *PRKN* and *PACRG* were identified on separate chromosomes and while the *PACRG* introns were consistently super-expanded *PRKN* introns are not. These findings suggest that proximity to *PACRG* or the associated genomic region may have contributed to the expansion of *PRKN* introns. Identifying elements involved in the expansion of the *PRKN*-*PACRG* locus may aid in understanding the underlying basis of the instability of FRA6E and the role of *PRKN*-proven genomic alterations in the aetiology of PD.

## POS-TUE-235

## NEURAL REMODELLING IN MICE WITH TARGETED ABLATION OF D1 DOPAMINE RECEPTOR-EXPRESSING STRIATAL NEURONS: A MODEL OF BASAL GANGLIA NEURODEGENERATION

Kim H.A.<sup>1</sup>, Jiang L.<sup>1</sup>, Parish C.L.<sup>1</sup>, O'Tuathaigh C.<sup>2</sup>, Waddington J.L.<sup>2</sup>, Ehrlich M.E.<sup>3</sup>, Lawrence A.J.<sup>1</sup> and Drago J.<sup>1</sup><sup>1</sup>Howard Florey Institute, Parkville, Victoria. <sup>2</sup>Royal College of Surgeons in Ireland, Dublin 2, Ireland. <sup>3</sup>Mount Sinai School of Medicine, New York, New York.

**BACKGROUND:** Dopamine responsive medium spiny neurons in the striatum are preferentially lost in Huntington's (HD) and Parkinsonian syndrome. A transgenic mouse line with selective ablation of dopamine receptor D1+ striatal neurons was generated using the Cre-LoxP system under the control of DARPP-32 (dopamine and adenosine 3',5'-cyclic monophosphate (cAMP)-regulated phosphoprotein, 32kDa) promoter. **METHODS:** Brain tissues from adult mice (WT n=7, MUT n=7) were analysed for Drd1, Drd2 and related neurochemical markers using *in situ* hybridization and immunohistochemistry. Tissues were immunolabelled for astrocytes (GFAP), medium spiny neurons (DARPP-32, CB, PPE), interneurons (GABA, NPY, VACht), dopaminergic cells (TH) and terminals (DAT). The concentration of extracellular dopamine and DOPAC was measured by HPLC. **RESULTS:** Striatal Drd1a, substance P, and dynorphin mRNA expression was reduced uniformly throughout the entire rostrocaudal extent of the dorsal striatum, while Drd2 and enkephalin mRNA was upregulated. Atrophy and astrogliosis was present in the striatum but not in the cortex and only striatal DARPP-32+ cells were reduced. CB+ cells were reduced, while GABA+/NPY+ interneurons and PPE+ projection neurons were increased and DAT+ dopaminergic terminals were reduced in the striatum. Dopamine concentration was reduced in the striatum and cortex, but DOPAC concentration was unchanged. **CONCLUSION:** Selective ablation of Drd1a-expressing striatal neurons demonstrated compensatory upregulation of Drd2-mediated indirect pathway and neural remodelling in a subset of striatal neurons.

## POS-TUE-234

## CRITICAL ROLE FOR THE PARAVENTRICULAR THALAMUS IN COCAINE-PRIMED 'RELAPSE': MODULATION BY COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT

James M.<sup>1,2</sup>, Jones E.<sup>1,2</sup>, Charnley J.<sup>1,2</sup>, Yeoh J.<sup>1,2</sup>, Levi E.<sup>1,2</sup>, Flynn J.<sup>1,2</sup>, Smith D.<sup>1,2</sup> and Dayas C.<sup>1,2</sup><sup>1</sup>School of Biomedical Sciences and Pharmacy, Centre for Brain and Mental Health Research, University of Newcastle. <sup>2</sup>HMRI, NSW, Australia.

**Purpose:** Recent studies suggest that the paraventricular thalamus (PVT) is involved in the reinstatement of alcohol-seeking (relapse-like behaviour). The present study aimed to extend these findings and investigate whether the PVT might also mediate cocaine-seeking behaviour. Further, hypothalamic neurons expressing the neuropeptide *cocaine- and amphetamine-regulated transcript* (CART) densely innervate the PVT and have been implicated in drug-motivated behaviours. It is not known, however, whether CART signaling within the PVT plays a role in the relapse phase of the addiction cycle. Thus, the effect of PVT-directed CART injections on cocaine-primed drug-seeking was determined. **Methods:** Rats were trained to self-administer cocaine and were then extinguished to a set criterion. Following extinction training, animals received either PVT-directed saline (n=9), tetrodotoxin (TTX, 5ng/0.25µl; n=7), or CART (1.25µg/0.25µl; n=5) followed by a cocaine-priming injection (10mg/kg, i.p.). A second group of rats received PVT-directed CART (0.5µg/0.25µl, n=4; or 1.25µg/0.25µl, n=4) or saline injections in the absence of a cocaine-priming injection. **Results:** Following a cocaine-prime, TTX-treated animals exhibited significantly attenuated drug-seeking behaviour (p<.05) compared to saline treated rats. PVT-directed CART injections significantly potentiated cocaine-seeking behaviour (p<.01). In contrast, treatment with either dose of CART alone had no effect on drug-seeking behaviour (p's>.05). **Conclusions:** These data suggest that the PVT plays a critical role in modulating cocaine-primed reinstatement of drug-seeking behaviour and that CART signaling in this region contributes to this response. Together, the present findings indicate that whilst CART plays a role in relapse-like behaviour, it is insufficient to produce a recovery of responding on its own.

## POS-TUE-236

## THE HUMAN Q133K TDP-43 MUTATION, BUT NOT THE WILD-TYPE TDP-43, INHIBITS NGF-MEDIATED DIFFERENTIATION OF PC-12 CELLS - MIMICKING THE EFFECTS OF SILENCING TDP-43 EXPRESSION

Tarco N., Rogers M.-L., Rush R.A. and Muyderman H.

Centre for Neuroscience, Flinders Medical Science and Technology, School of Medicine, Flinders University, SA.

The TAR-DNA-binding protein TDP-43 has recently been detected in cytosolic ubiquinone-positive inclusions in several neurological diseases and mutations in the gene encoding for this protein can cause an autosomally dominant inherited form of motor neuron disease. The normal function of TDP-43 suggests that modifications to this protein could impair neuronal plasticity. In this study we investigated the consequences of introducing the human TDP-43 mutation Q133k on NGF-mediated differentiation in PC-12 cells. The outcome of these experiments was compared to the response to NGF in cells overexpressing human wild-type TDP-43 and to cells in which TDP-43 expression had been silenced using a microRNA generating plasmid. PC-12 cells were transfected using Nucleofection. This approach resulted in 81 ± 4 % of the cells expressing the transgene (n=3). Differentiation was defined as having one or more neurites exceeding two times the diameter of the cell body. Exposure to 200 ng/ml NGF resulted in neurite outgrowth in 76 ± 11% of the cells at 24 hours and 86 ± 9% after 48 hours (n=3). In cells expressing the Q133k mutation, the proportion of differentiated cells was decreased by 60 ± 2 % compared to mock transfected cells (p<0.001, Student T-test, n=3). Overexpression of wild type TDP-43 did not affect differentiation. The effect of the Q133k mutation on NGF-mediated differentiation was mimicked by silencing TDP-43 expression. In those experiments, neurite outgrowth was inhibited by over 80% compared to controls (p<0.001; ANOVA; n=3). Together these results suggest that the observed effect of the Q133k mutation resulted from a lack of function of TDP-43.

## POS-TUE-237

### THE ANXIOLYTIC COMPOUND BNC210 EXHIBITS AN ANTIDEPRESSANT EFFECT WITHOUT SYMPTOMS OF PHYSICAL DEPENDENCE

O'Connor S.M.<sup>1</sup>, Andriambeloson E.<sup>2</sup>, Huyard B.<sup>2</sup>, Wagner S.<sup>2</sup> and Kremmidiotis G.<sup>1</sup>

<sup>1</sup>Bionomics Limited, 31 Dalgleish Street, Thebarton, South Australia, 5031. <sup>2</sup>Neurofit SAS, Bioparc, Parc d'Innovation, Boulevard Sebastien Brant 67400 ILLKIRCH, FRANCE.

BNC210 is a novel compound that displays acute anxiolytic activity in three rodent species and several models of anxiety. BNC210 is free of sedative side effects and does not cause abuse liability or development of tolerance. Here we present data to show that, in addition to its anxiolytic effects, acute administration of BNC210 at 100 mg/kg produces an antidepressant effect in the rat Forced Swim Test (FST), the primary screening test for antidepressants. Increased efficacy in this test was observed when BNC210 was dosed daily for 14 days, with significant antidepressant action seen at 30 mg/kg/day. Sudden discontinuation of antidepressant medication may produce withdrawal effects caused by physical dependence to the drug. In the 5 days following the chronic dosing period, rats were observed for changes in food intake, body weight and body temperature. Abrupt withdrawal of BNC210 did not produce any changes in these parameters indicating that it does not produce physical dependence and supporting its suitability for chronic use to treat anxiety and depression. Many antidepressant drugs have been shown to increase neurite outgrowth. At 0.01 nM and higher concentrations, BNC210 was found to enhance neurite outgrowth from rat primary cortical neurons. The concentrations at which BNC210 exerts its effects in this system correlate with its *in vivo* potency and measured brain concentrations.

## POS-TUE-239

### CANINE SAND MAZE: A NON-AVERSIVE SPATIAL MEMORY RETENTION TASK FOR USE IN A CANINE MODEL OF ALZHEIMER'S DISEASE

Salvin H.E.<sup>1</sup>, Valenzuela M.J.<sup>2,4</sup>, Sachdev P.S.<sup>2,3,4</sup> and McGreevy P.D.<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Science (B19), University of Sydney, Camperdown 2006. <sup>2</sup>School of Psychiatry, University of New South Wales, Sydney. <sup>3</sup>Neuropsychiatric Institute, Prince of Wales Hospital, Randwick NSW 2031. <sup>4</sup>Brain and Ageing Research Program, Faculty of Medicine, University of New South Wales, Sydney.

Canine cognitive dysfunction (CCD) is an age-related cognitive behavioural syndrome in older dogs. Pathological similarities with human dementia and the superior prediction of pharmacological responses have heightened interest in CCD as a model for human Alzheimer's disease. A major limitation to further research is the lack of a quick and accurate spatial memory test as current methods require in excess of 40 days of training. Experimental set up was based on a non-aversive appetitive appropriation of the Morris Water Maze. A 4.5m diameter circular pool was filled with sand/powdered food reward mix to a depth of 10cm. A food reward was positioned in a static location for all learning trials. Dogs were given 4 habituation trials followed by 16 learning trials which alternated between the reward being half buried and fully buried to a depth of 4cm. After a 90 minute break a probe trial was conducted in which the reward was buried 1/4 rotation around the pool. Time to learned annulus for healthy old (>8yrs, n=11) and young (1-4yrs, n=11) breed-matched dogs was compared. Average probe times were 6.88s and 38.53s for young and old dogs respectively (p=0.017). After correction for differences in motivation and learning times, probe times remained significantly different between groups (p=0.031). The Canine Sand Maze is a quick and non-aversive tool for determining short-term spatial memory in dogs. It is sufficiently sensitive to detect differences in memory function between young and healthy aged dogs.

## POS-TUE-238

### CHARACTERIZATION OF TERTIAPIN-Q ACTIVITY ON INWARDLY RECTIFYING K<sup>+</sup> CHANNELS

Pera E.<sup>1</sup>, Yow T.T.<sup>1</sup>, Absalom N.<sup>1</sup>, Johnston G.A.R.<sup>2</sup>, Hanrahan J.R.<sup>1</sup> and Chebib M.<sup>1</sup>

<sup>1</sup>Faculty of Pharmacy, University of Sydney, Camperdown, NSW 2006.

<sup>2</sup>Department of Pharmacology, University of Sydney, Camperdown, NSW 2006.

A large number of cells including cardiac myocytes and neuronal cells express the inwardly rectifying K<sup>+</sup> channels (Kir3.x or GIRK). This family of K<sup>+</sup> channels plays a pivotal role in regulating neuronal excitability and heart rate. GIRK channels can be modulated by several G-protein coupled receptors (GPCRs) including opioid, adrenergic, muscarinic, dopaminergic and GABA<sub>B</sub> receptors. The activation of GIRK channels involves many intrinsic factors including Mg<sup>2+</sup> and Na<sup>+</sup>, pH, phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) and intracellular proteins such as G $\beta$ o proteins. It has been shown that Tertiapin-q (TPNq), a peptide derived from honey-bee toxin venom, inhibits two members of the inwardly rectifying K<sup>+</sup> channel superfamily, the GIRK1/4 and the ROMK1 channels, with nanomolar affinities. In contrast, naringin, a flavonoid glycoside, is an activator of the GIRK1/4 channels. We hypothesize that naringin and TPNq bind to the same site on GIRK1/4 channels. To test this hypothesis, the effects of naringin at concentrations ranging from 10 $\mu$ M-1mM were evaluated in the presence of a constant concentration of TPNq (3nM) on *Xenopus* oocytes expressing wildtype GIRK1/4 channels. Channel activity was measured using two-electrode voltage clamp recordings. Results indicated that the response of naringin in the presence of TPNq showed a shift to the right compared with the activity of naringin alone. This indicates a possible competitive inhibition by TPNq against naringin. To determine whether TPNq can cause a right-ward shift of the EC<sub>50</sub> of GABA on GABA<sub>B</sub> receptor, oocytes expressing both GABA<sub>B(1b,2)}</sub> and GIRK1/4 were evaluated. TPNq inhibited the maximal GABA (100  $\mu$ M) response by 30% while the EC<sub>50</sub> concentration of GABA (3  $\mu$ M) was unaffected, indicating a non-competitive inhibition. We further explored the binding site of TPNq on GIRK channels. For these studies the homomeric GIRK1<sup>F137S</sup> mutant was expressed in oocytes and the effect of TPNq was evaluated. It was found that TPNq had a significant lower affinity on GIRK mutants compared to wildtype GIRK1/4 channels. 3 $\mu$ M of TPNq inhibits 50% of the barium sensitive current compared to 10nM necessary for GIRK1/4 wildtype. Further GIRK1 mutants will be studied to determine which amino acids are important for TPNq activity on GIRK1 and whether these mutations also affect naringin's activity.

## POS-TUE-240

### DEVELOPMENT OF EPHA4 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CNS INJURY

Dixon K.J.<sup>1</sup>, Roberts K.<sup>2</sup>, Chan J.L.<sup>2</sup>, Hughes R.A.<sup>2</sup> and Turnley A.M.<sup>1</sup>

<sup>1</sup>Centre for Neuroscience, University of Melbourne. <sup>2</sup>Department of Pharmacology, University of Melbourne.

**Purpose:** Robust regeneration of the human CNS after neurotrauma rarely, if ever occurs and there is no effective drug treatment. Following spinal cord injury, EphA4<sup>-/-</sup> mice undergo axonal regeneration with functional improvement. EphrinA5 is a ligand of EphA4, therefore we investigated whether ephrinA5 mimetic peptides can block EphA4 activity *in vitro* and *in vivo*. **Methods:** Peptides based on the ephrinA5 CD and GH loop structure were synthesised and tested. **Phosphorylation assay:** cortical astrocytes (n=3) were treated with peptide for 15 mins followed by soluble ephrinA5-Fc for 20 mins. EphA4 phosphorylation was quantitated by Western analysis. **Growth cone assay:** hippocampal neurons (n=3) were treated with peptide for 30 mins, +/- ephrinA5-Fc to induce growth cone collapse and the percent of neurites with growth cones determined. **In vivo administration:** following spinal cord hemisection in adult wild type mice (in each group, n=7), a GH loop peptide was administered IP for two weeks, followed three weeks later by anterograde tract tracing and motor function testing. **Results:** GH loop peptides were partially effective at blocking EphA4 phosphorylation, dependent on the peptides length and structure. CD loop peptides alone were ineffective at blocking phosphorylation, but when combined with GH loop peptides they were more effective than GH loop peptides alone. While some GH loop peptides inhibited ephrinA5-Fc induced growth cone collapse, they were ineffective at inducing regeneration or improving motor function following spinal cord injury, due to clearance in the kidneys. **Conclusion:** Ephrin mimetic peptides partially block EphA4:ephrin interactions, but their small size prevents them from reaching the CNS injury site *in vivo*.



## POS-TUE-241

**GENOTYPE-PHENOTYPE INTERACTION IN CHINESE MALE HEROIN-DEPENDENT SUBJECTS**Ho A.M.C.<sup>1,2</sup>, Cheung B.K.L.<sup>3</sup> and Stadlin A.<sup>1,4</sup><sup>1</sup>Dept of Anatomy, Chinese University of Hong Kong, Hong Kong. <sup>2</sup>Dept of Psychiatry, University of Queensland, Australia. <sup>3</sup>Substance Abuse Assessment Clinic, Kwai Chung Hospital, Hong Kong. <sup>4</sup>Dept of Anatomy, Chungbuk National University, South Korea.

The objective of this study is to compare cold-pain response (pain threshold, tolerance and net-pain tolerance) among current opioid users (n=48), long-term opioid abstiners (at least 1 year abstinence; n=34), and healthy controls (n=63). All subjects were male Chinese. We further investigated whether there is any phenotype-genotype interaction amongst these subjects in studying personality traits (neuroticism and extraversion measured by NEO PI-R) and gene polymorphisms of the opioidergic and dopaminergic systems. Results showed that pain threshold of ex-users resembled those of current users and their pain tolerance matched that of controls, resulting in a net tolerance (time that pain is endured) that fell between these two groups. The overall Neuroticism score was highest in current users compared to the other two groups ( $p = 0.0001$ ), with the subscale N3:Depression ( $p < 0.0001$ ) and N6:Vulnerability ( $p = 0.0002$ ) being the main contributory factors. Extraversion score of current users were lower ( $p = 0.048$ ) than the controls. COMT val158met polymorphism was shown to have a significant interaction ( $p = 0.007$ ) with the 'N3:Depression' subscale of Neuroticism in the current users. The present study observes that chronic opioid use causes cold-pain hyperalgesia in current users, which may be explained by their highly neurotic personality, and further supports the notion of pain-response recovery in former opioid addicts is a prolonged process that may take months to re-establish. This negative affect may be in part due to the influence of the COMT val/met genotype of these subjects. Pain management focused in the first 6 to 12 months of opioid abstinence may be an effective measure to prevent opioid-induced hyperalgesia-related relapse.

## POS-TUE-242

**LIFETIME MENTAL ACTIVITY PROMOTES STRUCTURAL AND FUNCTIONAL BRAIN INTEGRITY**Suo C.<sup>1</sup>, Fiatarone Singh M.<sup>3,4</sup>, Sachdev P.<sup>1,2</sup>, Wen W.<sup>1,2</sup>, Singh N.<sup>3,5</sup>, Brodaty H.<sup>1,6</sup>, Baune B.T.<sup>7</sup>, Gates N.<sup>1</sup> and Valenzuela M.<sup>1,2</sup><sup>1</sup>School of Psychiatry, UNSW. <sup>2</sup>Neuropsychiatric Institute, Prince of Wales Hospital, Australia. <sup>3</sup>Faculty of Medicine, University of Sydney. <sup>4</sup>Faculty of Health Sciences, University of Sydney. <sup>5</sup>Centre for Strong Medicine Balmain Hospital. <sup>6</sup>Primary Dementia Collaborative Research Centre UNSW. <sup>7</sup>Psychiatry & Psychiatric Neuroscience School of Medicine and Dentistry James Cook University.

Lifespan mental activity (LMA) is an important modifiable protective factor in the development of dementia. Individuals with a rich history of education, occupational complexity and cognitive life style activities are almost one-half the risk for developing dementia. The Life\_Experience\_ Questionnaire (LEQ) was developed and validated to quantify these lifestyle characteristics. This is the first study to correlate whole-brain structural or functional MRI with LMA on mild cognitive impairment individuals. Subjects (N=23, aged 65 to 80, 27.8% male) comprised an initial subsample of the SMART (Study of Mental Activity & Regular Training for the Prevention of Cognitive Decline in At Risk Older Individuals). LEQ scores were normally distributed (mean = 94.1+/-18.5). Those in the bottom (N=9) and top tertile (N=9) were categorized as Low and High LEQ respectively. High LEQ individuals exhibited several areas of reduced brain atrophy including temporal and frontal regions ( $p < 0.05$ ). There were also differences in the topographical pattern of hippocampal functional connectivity. Further, we observed areas of significantly increased connectivity with the posterior cingulate appeared in several regions in the High LEQ group. So LMA promotes a series of brain changes in the older brain. Some structural differences may account for decreased functional connectivity. To sum up, LMA may therefore lead to a structurally healthier and functionally more efficient brain in later life.

## POS-TUE-243

**MULTISENSORY FACILITATION AS A FACTOR INFLUENCING IQ IN CHILDREN**Barutchu A.<sup>1,2</sup>, Crewther S.G.<sup>1</sup>, Fifer J.<sup>1</sup>, Shivdasani M.<sup>2</sup>, Innes-Brown H.<sup>2</sup>, Danaher J.<sup>1</sup>, Toohey S.<sup>1</sup> and Paolini A.G.<sup>1</sup><sup>1</sup>School of Psychological Sciences, La Trobe University, Plenty Rd. Bundoora, VIC 3086 Australia. <sup>2</sup>The Bionic Ear Institute, 384-388 Albert St, East Melbourne VIC 3002, Australia.

The ability to consolidate multiple sensory inputs into a unified percept is imperative to perceptual learning and the acquisition of cognitive and intellectual abilities. This study investigated whether multisensory integration and its facilitative effect on motor actions is related to the intellectual abilities of children. The Wechsler Intelligence Scale for Children (WISC-IV) was used to assess the general intellectual abilities of 90 children, who showed good or poor multisensory facilitation during an audiovisual detection task when performed in quiet conditions and in the presence of auditory background noise. All children included in the study had Full-Scale IQs (FSIQ) above 80. Children who demonstrated good multisensory facilitation in quiet and noise showed above average FSIQs ( $p < 0.01$ ), while those with poor multisensory facilitation in noise showed significantly lower FSIQs and Verbal Comprehension Index (VCI) scores. Stable and consistent multisensory facilitation across quiet and noisy conditions is likely to optimise the ability to form appropriate associations between sounds and corresponding objects or events, leading to heightened general intellectual abilities, as assessed by the WISC-IV. Interestingly, in some children characterised as having slow motor responses, the likelihood of gain from multisensory integration improved in the presence of auditory background noise.

## POS-TUE-244

**EVIDENCE OF A ROLE FOR INTERSTITIAL CELLS OF CAJAL IN REGULATING NORADRENERGIC PERISTALSIS IN GUINEA-PIG VAS DEFERENS**

King D.A., Chung E.S.Y., Sanai F., McParland B.E. and Lloyd H.G.E.

School of Medical Sciences (Pharmacology), The University of Sydney, NSW 2006.

Sperm transport through the vas deferens requires coordinated, unidirectional contractile activity, but direct evidence of such peristalsis is scarce. It is possible that elevated intraluminal pressure stimulates rhythmic contractions in vas deferens, hence, we used pressure myography to examine the mechanisms underlying peristalsis in this tissue. Using guinea-pig prostatic vas deferens, rhythmic pressure changes that resembled peristalsis were recorded only when tissues were simultaneously stimulated with phenylephrine, an  $\alpha_1$ -adrenoceptor agonist, and increased intraluminal pressure (from 0 to approximately 20 cmH<sub>2</sub>O, achieved by fluid injection). The removal of either phenylephrine or pressure caused rhythmic activity to cease ( $n = 4$  tissues). Prazosin (1  $\mu$ M), an  $\alpha_1$ -adrenoceptor antagonist, reduced the amplitude of rhythmic contractions by approximately 60% ( $n = 4$  tissues), but did not reduce frequency. We have previously reported that imatinib (50  $\mu$ M), which disrupts the pacemaker function of interstitial cells of Cajal (ICC) in the gastrointestinal system, also inhibits pressure-dependent rhythmic contractions in isolated vas deferens ( $n = 7$  tissues). Further analysis of those tissues where some rhythmic contraction persisted ( $n = 4$  tissues) revealed that the frequency of contractions, but not the amplitude, was reduced, consistent with an effect on pacemaker cells. Importantly, we have demonstrated that imatinib does not reduce the amplitude of direct contractile responses to phenylephrine, indicating little or no effect on L-type Ca<sup>2+</sup> channels ( $n = 4$  tissues). Taken together, these results suggest that imatinib-sensitive ICC play a role in determining the frequency of noradrenergic peristalsis in vas deferens. Further experiments are warranted to explore the relevance of ICC in male fertility.

## POS-TUE-245

**HFI-1 REDUCES DAMAGE, BEHAVIOURAL DEFICITS AND NEUROPATHIC PAIN IN A RAT MODEL OF SPINAL CORD CONTUSION**

Weston R.M. and Jarrott B.

Howard Florey Institute, The University of Melbourne, Australia.

Spinal cord injury (SCI) predominantly occurs in young adults leading to permanent paraplegia and quadriplegia, and increased costs to society. Previously we have demonstrated that HFI-1, a mexiletine analog that has a sodium channel blocking pharmacophore linked to an antioxidant moiety, reduces deficits in model of mild SCI. We have subsequently assessed its efficacy in a model of spinal cord contusion. Male Hooded Wistar rats were anaesthetised (2% isoflurane/98% oxygen), and laminectomy performed at spinal level T10. Contusion injury was produced using the Infinite Horizon spinal cord impactor with a 2.5 mm diameter impounder dropped from a height of 6 mm at a force of ~150 kdyn. Mexiletine (12.5mg/kg, i.p.; n=7), HFI-1 (6mg/kg; 30mg/kg, i.p.; n=8) or vehicle (n=9) were administered at 3h after the injury and twice daily thereafter for 7 days. Behavioural tests were conducted weekly. At 6 weeks, rats were anaesthetised and transcardially perfused, to fix the spinal cords. Sections were cut and processed to examine the size of the cyst and modulatory effects of HFI-1 on lesion formation. HFI-1 treatment significantly decreased behavioral deficits as assessed by BBB scale, ladder walking test and inclined ledge beam ( $P<0.05$ ). Additionally, we utilised the plantar test to assess neuropathic pain, and observed mexiletine and HFI-1 treatment to reduce thermal hyperalgesia at 21 days post-SCI ( $P<0.01$ ). Mexiletine and HFI-1 reduced volume of damage following SCI by ~25% and ~45%, whilst axonal damage, assessed by sera phosphorylated neurofilament-H levels, was significantly reduced following HFI-1 (~70%) and mexiletine (~30%) treatment. These data indicate that HFI-1 may be a potential neuroprotective drug for the treatment of SCI.

## POS-TUE-247

**ALTERATION IN TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS FUNCTION IN A MODEL OF CHRONIC PAIN**Castro J.<sup>1</sup>, Martin C.M.<sup>1</sup>, Harrington A.M.<sup>1</sup>, Hughes P.A.<sup>1</sup>, Blackshaw L.A.<sup>1,2</sup> and Brierley S.M.<sup>1,2</sup><sup>1</sup>Nerve-Gut Laboratory, Hanson Institute, RAH, SA, Australia.<sup>2</sup>University of Adelaide, SA, Australia.

**Introduction:** Deletion of TRP-V1, -V4 and -A1 channels reduce mechanosensitivity of healthy colonic afferents (1) however; their role in visceral hypersensitivity is unknown. **Aims:** To determine the relative contribution of TRP-V1, -V4 and -A1 in colonic mechano- and chemo-sensation in a model of chronic post-inflammatory visceral hypersensitivity. **Methods:** Using an in vitro mouse colon preparation, we determined the effect of TRP channels agonists on mechano- and chemo-sensory function of splanchnic afferents. Mechanical hypersensitivity of high-threshold colonic afferents was induced by rectal administration of 0.1mL (130µg/mL) TNBS (2). Afferents were studied in control conditions or at 28 days post-TNBS administration when mucosal pathology had fully recovered. **Results:** (i) The TRPV1 agonist Capsaicin (3µM) caused desensitization to mechanical stimuli in both healthy and post-inflammatory afferents ( $n\geq 14$ ;  $P<0.05$  each). The chemosensory response to capsaicin was more intense and of shorter duration in TNBS recovery than healthy afferents. However, the number of fibres responding to capsaicin was greatly reduced in post-inflammation (37.8% mesenteric and 52.7% serosal). (ii) The TRPV4 agonist 5,6-EET (10µM) increased mechanosensitivity in healthy afferents ( $P<0.05$ ), this was not seen post-inflammation ( $n\geq 12$  each). The chemosensory response to 5,6-EET post-inflammatory was smaller than control afferents. (iii) The TRPA1 agonist AITC (40µM) increased mechanosensitivity in both healthy and post-inflammatory afferents ( $P>0.05$ ), with no major changes in chemosensory response ( $n\geq 15$  each). **Discussion:** Surprisingly TRPV1 channels are less functional in chronic visceral hypersensitivity, which contrast with the accepted role of TRPV1 in other pain models. TRPV4 channels contribute to mechanical hypersensitivity and post-inflammatory hyperalgesia, whilst TRPA1 plays an important mechanosensory role in multitude of conditions. <sup>1</sup>Brierley, 2009. <sup>2</sup>Hughes, 2009.

## POS-TUE-246

**ZIC3 REGULATES CRITICAL GENES INVOLVED IN THE ESTABLISHMENT OF THE NEUROECTODERM LINEAGE AND NEURONAL DIFFERENTIATION**Lim L.S.<sup>1</sup>, Hong H.F.<sup>1</sup> and Stanton L.W.<sup>1,2</sup><sup>1</sup>Stem Cell & Developmental Biology Group, Genome Institute of Singapore, Genome 02-01, Singapore 138672. <sup>2</sup>Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543.

The transcription factor *Zic3* (Zinc finger protein of the Cerebellum 3) is involved in a spectrum of brain development processes from the establishment of neuroectoderm (Nakata et al, 1997), to patterning of the dorsal neural tube (Aruga, 2004; Inoue et al, 2004; Purandare et al, 2002), development of mature dorsal neurons and axon targeting within the retina of the developing visual system (Zhang et al, 2004). However little is known to date about the *Zic3*-regulated networks involved in these processes. Our investigation into the functions of *Zic3* during early embryonic stem cell (ESC) neural differentiation has identified critical genes involved in brain development. We report that *Zic3* target genes include regulators of neurogenesis, CNS patterning, and neuronal function. Overexpression of *Zic3* in early ESC differentiation led to changes in transcript levels of these target genes (>2-fold; FDR<0.05). We further present a *Zic3* DNA-binding sequence by motif-discovery algorithms in promoter regions enriched by chromatin-immunoprecipitation, and demonstrate that promoter regions encompassing the *Zic3* DNA binding motif are functionally responsive to *Zic3* ( $p<0.05$ ). A detailed knowledge of *Zic3* transcriptional circuitry is fundamental to a comprehensive understanding of embryonic brain development and neuronal function in the adult brain. Our work has elucidated a set of *Zic3*-regulated genes that influence the critical decisions made during ES cell neural differentiation, and provide clues to pathways potentially regulated by *Zic3* in the establishment of neuroectoderm and embryonic brain development.

## POS-TUE-248

**IS THERE A DORSAL COLOUR AREA IN HUMAN VISUAL CORTEX?**Goddard E.<sup>1,3</sup>, Mannion D.J.<sup>1,3</sup>, McDonald J.S.<sup>1</sup>, Solomon S.G.<sup>2,3</sup> and Clifford C.W.G.<sup>1,3</sup><sup>1</sup>School of Psychology, The University of Sydney, Australia. <sup>2</sup>School of Medical Sciences, The University of Sydney, Australia. <sup>3</sup>Australian Centre of Excellence in Vision Science.

High resolution functional MRI enables the reconstruction of retinotopic maps in human visual cortex, but the organisation of these maps remains controversial in a number of areas. There is a current debate focussed on the human homologue of macaque V4, specifically whether V4 is divided between ventral and dorsal components, as in macaque, or whether there is an entire hemifield represented ventrally. Macaque V4 has been shown to be strongly responsive to colour. Here we compared responsivity to colour between the human ventral V4 and its putative dorsal component. We acquired high resolution functional images of human occipital cortex while participants ( $n=6$ ) viewed rotating wedge and expanding ring stimuli (standard stimuli for mapping visual field polar angle and eccentricity), and coloured vs black and white stimuli. We chose to use coloured vs black and white movie excerpts as more naturalistic stimuli than Mondrian patterns or gratings, which have been used by previous studies. We found a robust colour preference in ventral V4 and surrounding areas, and little or no colour preference in the vicinity of its suggested dorsal counterpart. Our results thus argue against the existence of a dorsal component of V4 and for a ventral representation of the entire hemifield. Furthermore, our results suggest that our stimulus is a useful localiser of ventral visual areas when used in conjunction with retinotopic mapping procedures.

## POS-TUE-249

**THE ROLE OF cAMP-RESPONSE-BINDING-ELEMENT PROTEIN (CREB) IN THE NUCLEUS ACCUMBENS IN RELAPSE TO METHAMPHETAMINE ADDICTION****Kraushaar N.J.**, Hunt L.R. and Cornish J.L.

Department of Psychology, Macquarie University, North Ryde, NSW, Australia.

**Rationale:** Methamphetamine abuse is a large problem within society however little is known about the underlying neurobiology that contributes to relapse to drug use. Recent studies have suggested a role for the cAMP-response-binding-element protein (CREB) in drug reward processes which can be activated by the release of cAMP-dependent-kinase PKA. Using the reinstatement model of drug-seeking behaviour, this study aimed to elucidate the role of the CREB activation within the Nucleus Accumbens (NAc) in mediating drug-induced relapse to methamphetamine-seeking behaviour. **Methods:** Male Sprague Dawley rats ( $374 \pm 6$ g,  $n=12$ ) were surgically implanted with a jugular vein catheter and bilateral cannulae into the NAc while under isoflourane anaesthesia. One week following surgery, rats were trained to self-administer intravenous methamphetamine at 0.1mg/kg/infusion on a fixed ratio schedule for 14 days. Following 14 days of behavioural extinction rats underwent 3 reinstatement test days where they were treated with an intracranial infusion of the PKA inhibitor Sp-cAMPs (10 nmol/0.5µl/side, 20 nmol/0.5µl/side) or aCSF (0.5µl/side) 30 minutes prior to a methamphetamine priming injection (1mg/kg, i.p.). **Results:** Treatment with the PKA inhibitor Sp-cAMPs produced a dose-dependent decrease in methamphetamine-induced drug seeking behaviour when compared to infusions of aCSF. **Conclusions:** This data suggests that PKA mediated activation of CREB in the NAc are involved in the relapse to methamphetamine use in rats.

## POS-TUE-250

**QUANTIFICATION OF NMDA RECEPTOR NR1 SPLICE VARIANT EXPRESSION IN THE CORTEX OF HUMAN ALCOHOLICS**Ng C.P., Ridge J.P. and **Dodd P.R.**

School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD 4072.

Ethanol is a modulator at the N-methyl-D-aspartate class of glutamate receptors (NMDAR) in the brain. NMDAR comprise a combination of NR1 and NR2 subunits. NR2 subunits are encoded by four different genes. In contrast, a single gene with a number of splice-variants encodes the NR1 subunit. These variants are generated by the absence or presence of exons 5, 21, 22 and 22' and influence the response of NMDAR to ethanol. In animal studies the receptor adapts to sustained ethanol exposure through altered expression of its subunits. We used real-time RT-PCR against engineered standards to assay the four C-terminal NR1 subunit mRNA splice variants in dorsolateral prefrontal and primary motor cortex tissue obtained at autopsy from chronic alcoholics with and without co-morbid cirrhosis of the liver (average daily ethanol consumption > 80 g), and from matched controls. Autopsies were performed under informed written consent by authorized pathologists for the Australian Brain Bank Network. The level of expression of the NR1-2 variant was significantly lower than that of all other variants, independent of area or pathology. The NR-3 and NR-4 variants were the most highly expressed. All variants were expressed at a markedly lower level in alcoholics than in controls in both areas. This was also true for the cirrhotic alcoholics, with the exception of the NR1-4 variant in the motor cortex, where levels were similar to controls. The data show that chronic alcoholism can influence the expression of NR1 subunits and that for these subunit isoforms, cirrhosis only has a minor influence.