# POSTERS

## POS-MON-001

### BONE MORPHOGENIC PROTEIN SIGNALLING ALTERS THE PRECURSOR CELL RESPONSE DURING CUPRIZONE-INDUCED DEMYELINATION

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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 oligodendrogliogenesis 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-002

### FRONTAL WHITE MATTER AND POSTERIOR CINGULATE ABNORMALITIES METABOLITE ABNORMALITIES IN OLDER AND CLINICALLY STABLE HIV+ INDIVIDUALS

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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 $^1$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 neuroinflamation 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-003

### AN AGE DIFFERENCE IN MU OPIOID RECEPTOR BINDING DENSITY IN THE HUMAN PUTAMEN BUT NO CHANGES IN SCHIZOPHRENIA

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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-004

### IMPACT OF ADULT VITAMIN $D_3$ (AVD) DEFICIENCY ON BRAIN FUNCTION AND BEHAVIOUR IN SPRAGUE-DAWLEY RATS

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**Purpose:** The incidence of schizophrenia varies across populations and is influenced by genetic and environmental factors. Vitamin $D_3$ exposure has been proposed as one such environmental risk factor and research in rodents indicates that prenatal vitamin $D_3$ deficiency affects brain development and behaviour. However, there is little evidence that vitamin $D_3$ 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 $D_3$ 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 $D_3$ 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 $D_3$ deficiency on the adult brain.

## POS-MON-005

### MODELLING COGNITIVE SYMPTOMS OF SCHIZOPHRENIA IN DEVELOPMENTAL VITAMIN D (DVD)-DEFICIENT RATS

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**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?

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**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

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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

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**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 affect 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

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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-010

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

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Epidemiological studies have suggested that schizoaphrenia 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-011

### ENVIRONMENTAL ENRICHMENT AFFECTS PCP-INDUCED BEHAVIOURAL AND NEUROCHEMICAL SYMPTOMS OF SCHIZOPHRENIA

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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-012

### CANNABINOID CB1 RECEPTOR DENSITY IS INCREASED IN THE DORSOLATERAL PREFRONTAL CORTEX (BRODMANN'S AREA 46) IN SCHIZOPHRENIA

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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 [$^3$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

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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 [$^{35}$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

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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 or14 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 serotoninergic 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

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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

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**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

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**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)[1] 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 ± SEM: BPD = 1.01 ± 0.52 vs. MDD = 1.17 ± 0.31 vs. Controls = 1.05 ± 0.31 ratio internal control; p = 0.62), 41kDA (BPD = 0.74 ± 0.34 vs. MDD = 0.99 ± 0.33 vs. Controls = 1.06 ± 0.51; p = 0.24), 47kDA (BPD = 0.74 ± 0.60 vs. MDD = 1.33 ± 0.87 vs. Controls = 1.36 ± 0.81; p = 0.20) and 50kDA (BPD = 2.02 ± 2.28 vs. MDD = 2.71 ± 1.96 vs. Controls = 2.73 ± 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. [1]Dean B et al (In Press) J.Affect.Dis. 10.1016/j.jad.2009.04.027 [doi].

## POS-MON-018

### ASTROCYTIC TUMOUR NECROSIS FACTOR UNDERLIES NEURON FUNCTION IN COGNITION

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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)α (GFAP-TNFα$^{+/+}$), prior to onset of behavioural deficits. These findings are compared directly to the hippocampal formation of a TNFα knock-out model (TNFα$^{-/-}$) 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 TNFα in hippocampal dependent learning and memory. **Methods:** Hippocampi from TNFα$^{-/-}$, GFAP-TNFα$^{+/+}$ and WT (n = 5) were subjected to indirect IHC for the analysis of TNFα levels and distribution in regions CA1, CA3 and the dentate gyrus (DG). **Results:** In GFAP-TNFα$^{+/+}$ there was a demonstrated accumulation of TNFα in hippocampal neurons prior to the onset of hippocampal-dependent behavioural deficits. GFAP-TNFα$^{+/+}$ mice also showed a significant increase in TNFα in regions CA3 and the DG ($p$ = <0.05) when compared to WT and TNFα$^{-/-}$ mice. WT mice demonstrated immunoreactivity of TNFα in regions CA1 and the DG. **Conclusion:** These findings suggest that astrocyte-produced TNFα is essential for normal development and functioning of the CA1 region of the hippocampus in cognitive processes. However, an overproduction of astrocytic TNFα 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-019

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

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**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-020

### SECETASE EXPRESSION AND NEUREGULIN 1 PROCESSING IN SCHIZOPHRENIA

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**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 TRIzol 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 n 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

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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

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**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

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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 ($p$'s≥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 $GABA_A$. 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

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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-γ, TNF-α, IL-1α, IL-1β, 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-α, 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-γ and TNF-α levels were decreased in PCP compared to saline treated animals. At weaning (PND21) IL-1α was decreased in the PCP-treated mice. A similar decrease in IL-1α 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

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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-026

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

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**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 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 (ΔSRI) and mice with complete deletion of ATM (ATM KO). **RESULTS**: Compared to wt mice, ΔSRI mice competently acquired a conditioned eyelid response ($p>0.05$, n=11 ΔSRI, 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±0.4% (mean±SEM, $p<0.0001$, n=7, ΔSRI), 18±0.3%($p<0.0001$, n=10, wt), and, 14±0.6% ($p<0.0001$ n= 8, ATM KO) 13±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-027

### GABAA RECEPTOR α3 CORTICAL EXPRESSION IN PERINATAL ASPHYXIA

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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. γ-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 $GABA_A$ receptor. If the $GABA_A$ 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 asphyxic newborn infants of late gestational age (Queensland Brain Bank). Ethical clearance for the project was obtained under Protocol Nº 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 $GABA_A$ receptor in frontal, motor, temporal and occipital cortex. $GABA_A$ receptor $\alpha_3$ protein was elevated in frontal, motor and temporal cortex of the human asphyxic 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-028

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

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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 α-internexin demonstrated an axonal sprouting response, with fine regenerative sprouts transversing 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 µm 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

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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

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**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-γ ($p<0.018$), TNF-α ($p<0.03$) 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-1β, IL-8 and TNF-α mRNA levels were increased ($p<0.001$) more than 6 hours after injury, with TNF-α 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

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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 % $O_2$) 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

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Allopregnanolone, a progesterone metabolite, is the most potent known modulator of the $GABA_A$ 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 ± 43 ng/mg) compared to non-injection controls (66.8 ± 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

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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-034

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

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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[1]. 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[2]. 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 µmol/kg) to the lesion site at a rate of 8µl/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 µmol/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-035

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

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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% $O_2$ in $N_2$ 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-1β (2.4±0.2) concentrations in the brain homogenates at 1d when compared with TAI+normoxia (IL-6: 8.3±0.6; IL-1β: 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-036

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

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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≥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

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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 $Mn^{2+}$ 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 $Mn^{2+}$-enhanced MRI (MEMRI), whereby rats receive $MnCl_2$ (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-038

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

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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% $O_2$) 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-039

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

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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-040

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

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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 vlPAG 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

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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±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-042

### IDENTIFICATION OF CELLULAR RESPONSES TO AUTOIMMUNE INJURY IN NEURONS

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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-043

### HYPOTHERMIA DURING TRANSIENT FOCAL ISCHAEMIA IN SPONTANEOUSLY HYPERTENSIVE RATS IS NOT NEUROPROTECTIVE

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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 (±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±45 v.106±27 $mm^3$, NS) or neurological scores at any time point, but, there was significant reduction in oedema volume (43±20 v. 25±20 $mm^3$, $P<0.05$). 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 $SpO_2$. 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-044

### ABLATION OF INSULIN-REGULATED AMINOPEPTIDASE GENE PROTECTS AGAINST ISCHEMIC DAMAGE IN THE BRAIN

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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

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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

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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-/-, CXCR2+/- 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-/- 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-/- 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-/- 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-/- 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 ledged beam test. Surprisingly, no improvement in neurological deficit was observed in CXCR2-/- mice compared to CXCR2+/- 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

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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 (Erondu,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

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**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 kynurenic 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

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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 $Gas6^{-/-}$ mice. We found an increased loss of oligodendrocyte-lineage cells in $Gas6^{-/-}$ mice compared with $Gas6^{+/+}$ mice following 14 days of cuprizone challenge (1502±139.0 vs 864.7±118.7 cells/mm$^2$ for $Gas6^{+/+}$ and $Gas6^{-/-}$ mice respectively; $p<0.001$). $Gas6^{-/-}$ mice also displayed an increased oligodendrocyte precursor cell (OPC) response compared with $Gas6^{+/+}$ mice (88.8±6.2 vs 122.8±18.6 cells/mm$^2$ for $Gas6^{+/+}$ and $Gas6^{-/-}$ 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-050

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

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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-051

### MODULATION OF BONE MORPHOGENIC SIGNALLING DURING DEMYELINATION

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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 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-052

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

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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 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

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**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-054

### GENE EXPRESSION PROFILING IN CANINE FUCOSIDOSIS

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The deficiency of α-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-055

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

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**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 TGFβ 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 ubiqutin 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-056

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

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Neurodegeneration of basal forebrain cholinergic neurons (BFCNs) is and 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 (0.4µg, 1µl) or the control toxin rabbit-IgG-saporin (0.4µg, 1µl) 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 affect 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

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**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 ΔFosB, 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 ΔFosB-positive cells and IBA-1-positive cells, in the infralimbic medial prefrontal cortex ($p$ <.05). Additionally, numbers of ΔFosB-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 ΔFosB after exposure to chronic stress may play a role in triggering stress-induced microglial activation and neuroinflammation in the medial prefrontal cortex.

## POS-MON-058

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

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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 $CB_1$ 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-059

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

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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 myriocin (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-060

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

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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

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**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

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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

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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-scapula 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

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Cannabis use has been shown to alter brain metabolism in both humans and animal models. [$^{18}$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 [$^{18}$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 [$^{18}$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 [$^{18}$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 in 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

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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-066

### PLASMA LEPTIN AND SALIVARY CORTISOL LEVELS ARE CORRELATED WITH STATE ALCOHOL AND SMOKING CRAVING IN EARLY ABSTINENT ALCOHOLICS

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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-067

### THE EFFECTS OF CHRONIC ADOLESCENT AND ADULT AMPHETAMINE TREATMENT ON PSYCHOTIC-LIKE BEHAVIOURS AND SYNAPTIC PLASTICITY

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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-068

### CALORIC VESTIBULAR STIMULATION REDUCES ALLODYNIA IN COMPLEX REGIONAL PAIN SYNDROME (CRPS) TYPE II

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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

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**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 $SOD1^{G93A}$ transgenic mouse model of motor neuron disease in-vivo. **Method**s: 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 $SOD1^{G93A}$ mice and adult $SOD1^{G93A}$ 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 $SOD1^{G93A}$ mice (n=3). Finally, MLR2-PEI-PEG-pGFP delivered into adult $SOD1^{G93A}$ 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 $SOD1^{G93A}$ mice.

## POS-MON-070

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

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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

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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/) 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

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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

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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-074

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

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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-075

### A HETEROGENEOUS STOICHIOMETRY OF α9α10 NICOTINIC ACETYLCHOLINE RECEPTORS IS DETECTED BY THE SELECTIVE CONOTOXIN VC1.1

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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 α9α10 heteromeric complex found in inner hair cells, dorsal root ganglion neurons and lymphocytes. The α9α10 receptor has previously been reported to form a stoichiometry of $(\alpha9)_2(\alpha10)_3$. The conotoxins Vc1.1 and RgIA are potent and selective inhibitors of acetylcholine-evoked currents in α9α10 receptors. We have investigated the stoichiometry of α9α10 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 α9 and α10 RNA injected into the oocytes from 1:1 to 10:1 α9:α10 (n ≥3 for each ratio). Furthermore, the biphasic nature of the curve is almost completely removed by "flooding" the injection ratio with α10 subunits at a ratio of 1:3 α9:α10. We interpret these results as demonstrating that the conotoxin Vc1.1 does not inhibit ACh-evoked currents when binding at the α9-α10 and α9-α9 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 $(\alpha9)_2(\alpha10)_3$ or the $(\alpha9)_3(\alpha10)_2$ stoichiometry *in vitro*.

## POS-MON-076

### INVESTIGATING THE NICOTINIC NON-COMPETITIVE BINDING SITE

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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)methyl2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate] contains an anthranilate ester side-chain displaying mixed competitive binding on α4β2, α3β4 nAChRs and competitive binding α7 nAChRs. Radioligand binding studies and X-ray crystallography has provided strong evidence that non-competitive binding sites exist at non-α 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 α4β2 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 β2 subunit, Loop D (N88, V89, W90, V91, K92, Q93, E94) of the α4 subunit and known accessible channel residues (2' T278, 6' S282, 9' L285, 13' V289, 16' L292) of the α4 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 β(-)α(+) interface and the competitive α(+)β(-) interface will be studied in the future.

## POS-MON-077

### EFFECTS OF LOBELINE, A NICOTINIC RECEPTOR LIGAND, ON THE CLONED CARDIAC $K^+$ CHANNELS, Kv1.5, Kv3.1 AND Kv4.3

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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 $IC_{50}$ value of 15.11 μM (n=7). The apparent binding ($k_{+1}$) and unbinding rate ($k_{-1}$) constants were 2.43 ± 0.22 μM$^{-1}$s$^{-1}$ and 40.92 ± 11.57 s$^{-1}$, respectively (n=7). The calculated KD value derived by $k_{-1}/k_{+1}$ was 16.86 μM. 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 (δ) 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 $IC_{50}$ value of 21.76 μM (n=6) and 28.25 μM (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

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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 α1 and α3 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 α1 and α3 GlyRs by automated patch-clamp electrophysiology. Results: This identified three Ircinidae sponges that yielded new examples of a rare class of glycinyl 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 α1 and α3 GlyR isoforms. One compound, strongly poteniated α1 GlyRs with an EC50 of 1.2±0.2 μM but inhibited α3 GlyRs with an IC50 of 7.0±0.5 μM (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 α1, α3 AND β SUBUNIT M2 DOMAINS

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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 α1 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 α3 and β subunits responded in a similar way to the α1 subunit during activation. GlyRs comprising mutated α1/β, and α3/β or α3 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 α3-R19'C GlyR exhibited a dramatically decreased glycine sensitivity ($EC_{50}$~12 mM; all results averaged from ≥ 5 cells). In contrast, α1/β-R19'C GlyR exhibited wild type-like glycine sensitivities. Fluorescence of the label attached to α3-R19'C GlyR increased by ~7% and the glycine fluorescence and current dose-responses overlapped. However, unlike α1 GlyR, the fluorescence response was slow to return to baseline after glycine removal. While fluorescence of α1/β-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 α3 and β subunit 19' residues are different to those experienced by the α1 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 Gβγ MODULATION OF THE α1 GLYCINE RECEPTOR

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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 βγ subunits (Gβγ). Gβγ 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 Gβγ modulation of human α1 homomeric GlyRs (α1GlyRs) expressed in HEK293 cells. Intracellular dialysis of 0.5 mM GTPγS during whole-cell recordings potentiated a glycine EC10 response by 119 ± 24% (n=5), with responses to a maximal glycine concentration remaining unaffected. This indicates that Gβγ 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 EC10 glycine concentration by 36 ± 2% (n=3). Interestingly maximal potentiation of α1GlyRs by GTPγS dialysis, did not occlude subsequent ethanol potentiation; co-application of 100 mM ethanol potentiated EC10 glycine responses after dialysis by 63 ± 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 Gβγ binding motifs, 385KK386, to glutamate, abolishes ethanol potentiation (2 ± 7%; n=3). Overall, our data support an interaction between the structural determinants of both Gβγ and ethanol modulation of α1GlyRs but also suggest that ethanol modulation is more complex than simply liberating Gβγ subunits.

## POS-MON-081

### CHARACTERISING A HIGH-THROUGHPUT ASSAY FOR DRUG SCREENING AT THE α2β2γ1 $GABA_A$ RECEPTOR

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The $GABA_A$ receptor is an important target for sedative and anxiolytic drugs. Recent work from our lab has shown that the γ1 subunit is highly expressed in the amygdala, and we propose that γ1-containing receptors play an important role in anxiety. To investigate this hypothesis, we wish to identify selective modulators of γ1-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 $GABA_A$ 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 γ1 subunit, we used zinc, a selective antagonist of γ-containing receptors. Three different ratios of α2:β2:γ1 DNA were trialled (1:1:3, 1:1:6, 1:1:9), as well as α2:β2 (1:1). Addition of zinc (100uM) reduced the quench in response to 10uM GABA by 94% in cells expressing α2:β2, compared to 44% in cells expressing α2:β2:γ1 (1:1:3). This difference was significant ($p<0.05$, ANOVA with Dunn's multiple comparison), however increasing the quantity of γ1 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 α2β2γ1 $GABA_A$ receptors.

## POS-MON-082

### EFFECT OF *GINKGO* TERPENOID LACTONES ON CYSTEINYL MUTANTS OF GABAA RECEPTOR PORE

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Clinical studies showed that the extract of *Ginkgo biloba* (EGb 761) reduced anxiety without causing sedation[1]. Anxiolysis without sedation of the extract active constituents bilobalide and ginkgolide A was also demonstrated in animal models[2,3]. 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 $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ 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 $\gamma_{2L}$ 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_1$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-083

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

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We previously employed a disulfide trapping approach in an attempt to determine how the pore-lining second transmembrane domains (M2) of γ-aminobutyric acid type A receptors (GABAARs) move to open the channel. The M2 domain T6' residue lines the pore, and we showed that α1/β1T6'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, α1/βT6'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, β subunit 6' cysteines move into sufficiently close proximity for disulfide formation via large random motions that appear to be a unique feature of β subunits. Because cross-linking of adjacent β 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 β subunits during gating.

## POS-MON-084

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

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Concentrations of glycine are regulated via the $Na^+/Cl^-$-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 (NALAla), N-arachidonyl-D-alanine (NADAla) and N-arachidonyl-γ-aminobutyric acid (NAGABA). Transport currents induced by application of glycine alone and in the presence of the NAAAs were measured (n≥5/transporter). GLYT2 (and not GLYT1) is inhibited by NAGly, NADAla and NAGABA whereas GLYT2(GLYT1EL2) and GLYT2(GLYT1EL4) had reduced sensitivities. Interestingly, GLYT2 and GLYT2(GLYT1EL2) are inhibited by NALAla whereas GLYT2(GLYT1EL4) is not. GLYT2R531L and GLYT2K532G had reduced sensitivity to NAGly ($IC_{30}$: 13±2 μM; 9±1 μM, respectively) compared to GLYT2 ($IC_{30}$: 3.4±0.6 μM) while GLYT2I545L had markedly reduced sensitivity to NAGly ($IC_{30}$: >30 μM). GLYT2R531L, GLYT2K532G and GLYT2I545L also had reduced sensitivities to NALAla ($IC_{30}$: 14±1 μM; 12±1 μM; and 21±1 μM, respectively) compared to GLYT2 ($IC_{30}$: 5.9±0.7 μM). In conclusion, EL2 and EL4 of GLYT2 are important in the selective inhibition of GLYT2 by NAGly, NADAla and NAGABA while only EL4 of GLYT2 is required for NALAla inhibition of transport. Key residues in GLYT2 EL4 required for NAGly and NALAla sensitivity are R531, K532 and I545.

## POS-MON-085

### EAAT5 MEDIATES GLUTAMATE TRANSPORT IN MOUSE VESTIBULAR EPITHELIUM

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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

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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+, 1H+, and the counter-transport of 1K+. 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, Pyroccoccus 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

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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

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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 ($B^0AT1$), 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 $B^0AT1$ 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 $B^0AT1$, and the mechanism by which this occurs. It was first discovered that APN increases the rate of $B^0AT1$ 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 $B^0AT1$ transport ($p \leq 0.05$), and an increase in the substrate affinity ($K_m$) of $B^0AT1$ ($p \leq 0.001$). The increase in maximum rate was shown to be due to an increase in surface expression of $B^0AT1$, indicating APN functions as a facilitator of $B^0AT1$ 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 $B^0AT1$ 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 $B^0AT1$. 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 $B^0AT1$ substrate affinity.

## POS-MON-089

### BIOLOGICAL ACTIVITY OF ALANINE-SUBSTITUTED ANALOGUES OF α-CONOTOXIN VC1.1 ON N-TYPE CALCIUM CHANNELS IN RAT SENSORY NEURONS

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α-Conotoxin Vc1.1 is a 16 amino acid disulfide peptide that is a selective antagonist of the α9α10 nicotinic acetylcholine receptor (nAChR) subtype but has recently been shown to be a more potent inhibitor of N-type $Ca^{2+}$ channel currents in dissociated neurons from rat dorsal root ganglia (DRG). The inhibition of N-type $Ca^{2+}$ channel currents was blocked by inhibitors of $G_{i/o}$ and selective $GABA_B$ receptor antagonists suggesting that Vc1.1 acted via $GABA_B$ receptors (Callaghan et al., 2008, *J. Neurosci.* 28:10943-51). To further explore the structure-activity relationship for Vc1.1 inhibition of N-type $Ca^{2+}$ 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 $Ca^{2+}$ 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 $Ca^{2+}$ 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 α9α10 nAChR whereas it is inactive at inhibiting N-type $Ca^{2+}$ channel currents. These findings contribute to an improved understanding of the molecular basis for the $GABA_B$ receptor-mediated inhibition of the N-type calcium channel current by Vc1.1.

## POS-MON-090

### TEMPORAL AND SPATIAL EXPRESSION OF SODIUM CHANNEL ALPHA-SUBUNITS AND SPLICE VARIANTS IN THE DEVELOPING C57BL/6 MOUSE BRAIN

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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-091

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

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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 hyperpolarization6activated, cation nonselective (HCN) channels. Surprisingly, the adenosine-induced inhibition was blocked by 3μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective A1 receptor antagonists(n=6), but not by 10μM 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 $Ca^{2+}$ channels and extracellular $Ca^{2+}$. 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-092

### IDENTIFICATION OF A LOSS-OF-FUNCTION POLYMORPHISM IN THE HUMAN P2X4 RECEPTOR

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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 ($EC_{50}$ of 192 μM compared to wild-type P2X4 $EC_{50}$ of 5 μM). 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

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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

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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 Ca2+ 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 Ca2+-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 Ca2+ 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

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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-/- 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-/- 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

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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 brains 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 solitarious 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

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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 isofluorane (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-098

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

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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 ε4allele (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$^2$). 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. [1] 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-099

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

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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 slowed, 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-100

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

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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

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In cardiomyocytes, calcium is released from SR intracellular stores through ryanodine receptors (RyR). RyRs are regulated by Ca2+ 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 β-adrenergic receptors (β-AR) by adrenaline and noradrenaline. β-ARs couple to Gsα-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 μM isoproterenol (β1-adrenergic agonist) or with Krebs alone (control). Hearts were rapidly frozen in liquid N2 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 Ca2+. The mean activity of RyRs from isoproterenol-stimulated hearts was 10-fold higher than control RyRs at diastolic [Ca2+] (100 nM) but was not significantly different at systolic [Ca2+] (>1 μM, 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

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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; 0.1–1μM) and depolarisation (10-120mM $[K^+]_o$). The selective L-type channel blocker, nifedipine (0.1, 1μM), substantially inhibited pressure-induced constriction, whereas the putative T-type channel blocker, mibefradil, inhibited myogenic tone at low concentration (1μM) but produced paradoxical constriction at high concentration (10μM). A mibefradil analogue, NNC-550396 (3, 10μM), 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 (1μM) followed by mibefradil (1μM). 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 $[K^+]_o$, that were not reliant on intracellular calcium (blocked by 10μM 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

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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. Ca2+ 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 ± 28 counts/sec/mg vs. control, 489 ± 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

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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, 10μM) compared to conventional L-type calcium channel blockade (verapamil, 10μM) 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 $K^+$-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 IP3 receptor and store-operated calcium channel inhibitor, 2-aminoethyl diphenylborinate (100μM) further attenuated the force ($p<0.05$; n=4). Following complete depletion of intracellular calcium using the sarco-endoplasmic reticulum calcium ATPase inhibitor, cyclopiazonic acid (10μM), ET-1-mediated contractile responses were almost completely abolished ($p<0.05$; n=4). Combining calcium channels blockers with protein kinase C inhibitor (5μM) also resulted in significant attenuation of ET-1-mediated vasoconstriction. In conclusion, extracellular, $IP_3$-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

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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 $Ca^{2+}$ homeostasis. The cardiac $Ca^{2+}$ 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 anthracyclines-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 $Ca^{2+}$ homeostasis and contribution to subsequent cardiotoxicity.

## POS-MON-106

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

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) causes sudden cardiac death due to mutations in the cardiac $Ca^{2+}$ 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 $Ca^{2+}$ loading of the SR leading to pro-arrhythmic oscillatory $Ca^{2+}$ release. RyRs were isolated from human and sheep hearts, and incorporated in artificial lipid bilayers to conduct single channel recordings under diastolic $Ca^{2+}$ conditions. Flecainide (10 μM, 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 μM cytoplasmic concentration) had no significant effect on mean open time but increased mean closed times. In CPVT mice ventricular myocytes, flecainide significantly reduced $Ca^{2+}$ 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 $Ca^{2+}$ leak or SR $Ca^{2+}$ content. In contrast, tetracaine decreased spark frequency and spark-mediated SR $Ca^{2+}$ leak, resulting in a significantly increased SR $Ca^{2+}$ content. We propose that smaller spark mass contributes to flecainide's antiarrhythmic action by reducing the probability of saltatory wave propagation between adjacent $Ca^{2+}$ release units. Hence, RyR2 open-state inhibition provides a new therapeutic strategy to prevent diastolic SR $Ca^{2+}$ waves and resulting triggered arrhythmia, like CPVT.

## POS-MON-107

### SPATIAL ASSOCIATION OF TRPC3, $IK_{CA}$ AND MYOENDOTHELIAL GAP JUNCTIONS IN RAT MESENTERIC ARTERY

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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 (Cxs), endoplasmic reticulum (ER) inositol 1,4,5-trisphosphate receptors, and intermediate conductance calcium-activated potassium channels ($IK_{Ca}$). 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 $IK_{Ca}$ and myoendothelial gap junction Cxs, consistent with potential for functional interaction. 1. Dietrich et al. J Biol Chem 2003; 278:47842-52.

## POS-MON-108

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

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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 μg protein/mg wet muscle, p=0.013). Western blot analysis showed that Tau supplementation increased calsquestrin 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 calsquestrin 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

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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-110

### DIMINISHED CARDIOVASCULAR BUT NOT EMOTIONAL REACTIVITY TO CONTEXTUAL FEAR CONDITIONING IN AT1A RECEPTOR KNOCKOUT MICE

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Our recent studies indicate that angiotensin $AT_{1A}$ receptor ($AT_{1A}^{-/-}$) 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 $AT_{1A}$ receptors on cardiovascular and behavioural effects of contextual fear conditioning in $AT_{1A}^{-/-}$ (n=6) and $AT_{1A}^{+/+}$ (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 $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice (+29±5 and +31±3 mmHg, respectively). Conversely, the BP rise during the re-exposure sessions was lower in $AT_{1A}^{-/-}$ than $AT_{1A}^{+/+}$ mice (+24±4 and +37±2 mmHg, respectively). However, immobility duration (freezing) during fear conditioning and extinction was similar between groups. Moreover, $AT_{1A}^{-/-}$ 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 $AT_{1A}$ 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-111

### CATESTATIN ATTENUATES THE EFFECTS OF INTRATHECAL NICOTINE AND ISOPROTERENOL

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Catestatin (Cts; human chromogranin $A_{352-372}$) 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-112

### EFFECT OF 5HT1A RECEPTOR ACTIVATION IN LOWER BRAINSTEM ON CARDIOVASCULAR AND BEHAVIOURAL RESPONSES TO PSYCHOLOGICAL AND PHYSICAL STRESS

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**Introduction:** In the conscious animal, the activation of the 5HT1A receptor via systemic injection of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a 5HT 1A 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 5HT1A 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 5HT1A 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 5HT1A 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

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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-114

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

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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 anesthetised 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-115

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

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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-116

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

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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

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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±0.16 mmHg (mean±SE), whereas Group 2 and 3 had significant increases in IAP to 3.1±0.38 mmHg ($P < 0.05$) and 3.8±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

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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

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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 ± 15.43 pA, n = 16 cells from 7 animals) (Control: -101.3 ± 13.98 pA, n = 14 cells from 6 animals), paired pulse ratio (EAN: 1.402 ± 0.061, n = 16 cells from 7 animals) (Control: 1.395 ± 0.082, n = 14 cells from 6 animals), or percentage inhibition by an $EC_{50}$ concentration of NAd (EAN: 44.15 ± 11.76%, n = 7 cells from 3 animals) (Control: 36.54 ± 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 ± 54.78, n = 10) (Control: 236.8 ± 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

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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 α-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 ± 4.47% and 41.32 ± 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 ± 4.76% and a 27.09 ± 1.84% increase in apical dendritic length ($P<0.05$) and increased branching (65 - 80µm from soma) compared to the control (30 - 50µm 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

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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-122

### CATECHOLAMINE NEUROTRANSMISSION IN THE ORBITAL FRONTAL CORTEX EVOKED BY STIMULATION OF THE VENTRAL TEGMENTAL AREA

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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 µA, 60 Hz) catecholamine release in medial regions of the OFC (0.5 mm lateral to midline) of urethane anesthetised 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-123

### TOPOGRAPHICAL SPECIFICITY OF RESPIRATORY REGULATION BY THE DORSOLATERAL PERIAQUEDUCTAL GREY

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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 ± 12 and 42 ± 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 ± 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-124

### INTENSITY-DEPENDENT BRAIN RESPONSES DURING CAPSAICIN INHALATION

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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

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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

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**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

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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

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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 GPF 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)

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Purpose: Tyrosine hydroxylase (*th*) is the rate-limiting enzyme in the biosynthetic pathway of the catecholamine neurotransmitter dopamine and its derivates. *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-130

### MOLECULAR CHARACTERISATION OF TARGETED PROLIFERATIVE CELLS

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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-131

### ZEBRAFISH NICASTRIN IS REQUIRED FOR MID AND HINDBRAIN DEVELOPMENT

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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*$^{-/-}$ (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*$^{-/-}$ is deficient in *ncstn*. Notably, our microarray data between zebrafish *ncstn*$^{hi1384}$ 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*$^{hi1384}$-Tg(*acinus*:GFP) line (n=137, 100%). The results of these analyses indicate that zebrafish *ncstn*$^{hi1384}$ could potentially be used as an animal model for the study of Alzheimer's disease.

## POS-MON-132

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

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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-regualted 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 preformed 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

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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-134

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

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**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 $O4^+$ 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 $HuNu^+$ 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-135

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

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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 ($TrkB^{fl/fl}$ MBP $cre^{+/-}$ 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-136

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

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**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 ($0.258 \times 0.258 \times 1\ mm^3$) diffusion MRI data (b-value = 1000 s/$mm^2$, 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

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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-tfi 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-138

### NDFIP1 EXPRESSION IN THE CEREBRAL CORTEX

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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-139

### CORTICAL DEVELOPMENT IN THE Tc1 MOUSE; A MODEL OF DOWN SYNDROME

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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 transchromosomic 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 diving 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-140

### NDFIP1 REGULATES CORTICAL NEURON NUMBERS AND IS REQUIRED FOR DENDRITIC COMPLEXITY

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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 β-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

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**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-142

### OLFACTORY PROGENITORS RESPOND DIFFERENTIALLY TO GDNF AND NEURTURIN

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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 (GFRαs) and two RET receptor tyrosine kinases. RET exists as two main isoforms, RET9 and RET51, potentially activating different biological pathways. GDNF binds preferentially to GFRα1 and NTN to GFRα2, although the reverse has been observed *in vitro*. We previously reported expression of GDNF, NTN, GFRαs and RET isoforms in olfactory neuroepithelium, a region of the nervous system characterised by ongoing neurogenesis. GFRαs showed differential expression, with mature olfactory neurones expressing GFRα1, and progenitors and immature neurones expressing GFRα2. In the current study we used an olfactory neurosphere culture system to define expression of the GFRαs 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. GFRα2 expression paralleled RET9 and GFRα1 was limited to more mature, peripheral cells. Based on these results, we propose GFRα2/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 GFRα2/RET9 complex.

## POS-MON-143

### EPHA4 INHIBITS NEURAL PRECURSOR PROLIFERATION IN THE ADULT HIPPOCAMPUS

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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 ± 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 ± 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 ± 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-144

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

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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 SUPRESSES NEURONAL DIFFERENTIATION IN ADULT NEURAL PRECURSOR CELLS

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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 $NAD^+$-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-146

### ISOLATION OF HUMAN NEURON RESTRICTED PRECURSOR CELLS FROM FETAL SPINAL CORD DERIVED HISTOTYPIC SPHERES

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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 (200-500µm diameter) significantly affect histotypic sphere formation; (v) NEB cytoarchitecture is characterized by a surface layer of neuron restricted precursor cells (NRP); and (vi) $BMPRII^+$NRPs were characterized as $nestin^-$/$vimentin^-$/$GFAP^-$/$NeuN^-$/MAP2a/$b^{-/+}$/$\beta III\text{-tubulin}^+$ 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 upregulatation 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 $BMPRII^+$NRP cells which could have application in human spinal cord injury.

## POS-MON-147

### THE MIGRATORY BEHAVIOR OF INDIVIDUAL NEURAL CREST-DERIVED CELLS IN THE EMBRYONIC GUT

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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 µm/hour, some individual cells migrating along pre-existing strands of the network migrated at ~100 µm/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-148

### DIFFERENTIAL GENE EXPRESSION IN MIGRATING CORTICAL INTERNEURONS DURING MOUSE FOREBRAIN DEVELOPMENT

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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

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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

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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 symptomology 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

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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

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**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

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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-154

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

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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-155

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

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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-156

### INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION IN THE PRIMARY OLFACTORY PATHWAY OF WILD TYPE, CX3CR1$^{+/GFP}$ AND CX3CR1$^{GFP/GFP}$ MICE FOLLOWING DAMAGE AND BACTERIAL CHALLENGE

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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$^{+/GFP}$ and CX3CR1$^{GFP/GFP}$ mice had a significantly lower density of macrophages in the glomerular and granular layers of the olfactory bulb. CX3CR1$^{+/GFP}$ mice had significantly more iNOS-expressing cells in the glomerular layer than CX3CR1$^{GFP/GFP}$ 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

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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 ($Y1^{-/-}$, $NPY^{-/-}$, $PYY^{-/-}$ and $NPYPYY^{-/-}$) 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 $NPYPYY^{-/-}$ and $PYY^{-/-}$ mice identified a significantly greater number of olfactory receptor neurons compared to WT, $Y1^{-/-}$ and $NPY^{-/-}$ mice ($p<0.0001$). Furthermore, $NPY^{-/-}$ 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, $Y1^{-/-}$, $NPY^{-/-}$, $NPYPYY^{-/-}$ and $PYY^{-/-}$ mice. The number of neurospheres that survive *in vitro* from $NPY^{-/-}$ are significantly reduced compared to WT controls at 3 weeks ($p<0.05$). Olfactory neurospheres from $NPYPYY^{-/-}$ and $PYY^{-/-}$ 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

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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

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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

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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 isofluorane, 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 mechanoceptor function.

## POS-MON-161

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

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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-162

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

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Canonical transient receptor potential (TRPC) non-selective cation channels assemble from TRPC subunits and exhibit multiple activation mechanisms. TRPC3 has been proposed as a $Ca^{2+}$ entry channel responsible for $Ca^{2+}$ 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 Reissners 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-163

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

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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-164

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

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**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

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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 synaptophysine 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-166

### $P2X_2$ AND VILIP1 CO-EXPRESSION IN THE MOUSE COCHLEA AND VESTIBULAR SYSTEM

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The association between the neuronal calcium sensor VILIP1 and the $P2X_2$ 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 $P2X_2$ knockout ($P2X_2^{-/-}$) mice were fixed in PFA and cryosectioned after decalcification. Floating sections were immunolabeled using anti-$P2X_2$ (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 $P2X_2$ was confirmed in the cochlear spiral ganglion and the vestibular Scarpas ganglion neurons. In addition, VILIP1 expression was also found in the cytoplasm of the epithelial cells of the organ or Corti, except the outer hair cells, pillar cells and Deiters cells. $P2X_2$ 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 $P2X_2$ was immunolocalized at the cuticular plates and also throughout the dark cells. In the utricle, VILIP1 immunolabeling was in the stereocilia, whereas $P2X_2$ 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-167

### SELECTIVE CHANGES IN EXPRESSION OF A POTASSIUM CHANNEL IN AN INNER EAR PUMPING EPITHELIUM

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K+ 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+ 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 strial function (Housley et al, 2002). We therefore tested the hypothesis that the reduction of K+ 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+ 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+ channel expression compared to controls. The mechanism of the observed selective reduction in strial K+ channel expression requires further investigation. Housley GD et al (2002) Audiol Neurootol. 27:55-61. Marcus DC, Shen Z. (1994) Am J Physiol Cell Physiol 267: C857–C864.

## POS-MON-168

### NEUROTROPHINS AND AUDITORY NERVE FUNCTION

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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

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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 Cl-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 $A_1$ adenosine receptor agonist CCPA. No threshold recovery was observed with CGS-21680 or Cl-IB-MECA, the selective $A_{2A}$ and $A_3$ 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 $A_1$ 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 $A_1$ adenosine receptors as prospective pharmacological targets to mitigate noise-induced cochlear injury.

## POS-MON-170

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

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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+/+) and mice lacking the neurotrophin-binding domain of p75NTR (p75-/-) 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-/- 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-171

### TRACKING THE EXPRESSION OF GAD-67 AND GABAA RECEPTOR α1 SUBUNIT IN MULTIPLE NUCLEI OF THE AUDITORY PATHWAY FOLLOWING NOISE-INDUCED HEARING LOSS

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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 $GABA_A$ receptor α1 subunit ($GABA_ARα1$) 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 $GABA_ARα1$ 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 $GABA_ARα1$ 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-172

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

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Inner ear inflammation is thought to contibute 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 (110dBSPL for 4hours) 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

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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

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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 neuro¬nal 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-lysin and Poly-D-lysin/ 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-422b, 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

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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*

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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. β-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

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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-178

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

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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-179

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

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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 stimluation locations confirmed using 3D modelling of X-Ray CT images, multiunit cluster response, and histology. This may reflect the complex mechansims of the cochlear nucleus, and suggest that further investigation into stimulation strategies is warranted.

## POS-MON-180

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

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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

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**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

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Thin-film microelectrodes were used to stimulate the cochlea of five urethane-anesthetised rats. Simultaeous 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 activition 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 electically-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 specifity in future cochlear implant devices.

## POS-MON-183

### *IN VITRO* EPIRETINAL STIMULATION USING A HEXAGONAL ELECTRODE ARRANGEMENT

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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

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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, 350us per phase, 414uA). 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.**[1, 2] and Protti D.P.[1, 2]
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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-186

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

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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-187

### INTRINSIC PHYSIOLOGICAL PROPERTIES OF RAT RETINAL GANGLION CELLS

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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-188

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

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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 $Na_v1.8$ 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 $Na^+$ currents, voltage-gated $K^+$ & $Ca^{2+}$ currents were suppressed using a $Cs^+$ based internal solution with 20 mM TEA and 0.1mM $CdCl_2$ 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

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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). $GABA_A$ 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)

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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$^2$) from $g_h$=$g_T$=0 to $g_h$=$g_T$=0.1S/cm$^2$. 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: $g_T$<=2.8*$10^{-5}$S/cm$^2$, $g_h$<=$10^{-5}$S/cm$^2$; OFF cells: 3.5*$10^{-4}$<=$g_T$<=1.1*$10^{-3}$S/cm$^2$, $g_h$<=$10^{-5}$S/cm$^2$. 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

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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 DiI 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, >65µm diameter) and narrow-field (12 types, <65µm diameter). The most common wide-field type (105µm diameter) was monostratified near the RGC layer. A wide-field bistratified type was present mainly in the peripheral retina. The most prevalent narrow-field type (44µm diameter) was monostratified 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 µm, 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

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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 $Na^+$ spikelets riding on top of a slower $Ca^{2+}$-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

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**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 indicies[1] (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≥0.40, "o-cells") similar to V1 cells[2]. 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±0.07 (mean±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±range: 0.96±1.47 cycles/deg, n=7) was similar to other koniocellular cells[2]. Relative low SF response ratios (mean±range: 0.22±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-194

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

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**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-195

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

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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-196

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

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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

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Intracellular recordings from identified neurons within the optic lobe of the dragonfly (*Hemicordulia tau*) reveal selectivity for small moving objects[1]. 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[2] 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[3] 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 $GABA_A$ RECEPTOR NUMBERS ARE REDUCED IN PURKINJE CELLS OF THE DYSTROPHIN-DEFICIENT mdx MOUSE

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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 $GABA_A$ receptors which occurs as a direct consequence of the absence of dystrophin from the post-synaptic density. In the present study have looked at the effect of an absence of dystrophin on the number and function of $GABA_A$ 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. Immunoflourescence 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 ± 2.95; n=14) compared to littermate controls (53.03 ± 4.11; n=12) ( p= 0.01). These electrophysiological findings were supported by the immunoflourescent assay, which showed a reduced density of $GABA_A$ 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 EXCITABILTY OF CORTICAL LAYER 5 PYRAMIDAL NEURONS VIA LOCATION-DEPENDENT MECHANISMS

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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 μM) 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 μM) 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 α1β2γ2 GABA-A RECEPTOR PROBED USING VOLTAGE-CLAMP FLUOROMETRY

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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 α1 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 α1, β2 and γ2 subunits, and in the M2-M3 linkers of the α1 and β2 subunits. We generated the α1-R186C, α1-N275C, β2-I205C, β2-K298C and γ2-S195C mutations. GABA-A receptors comprising mutated α1/β2 and α1/β2/γ2 subunits were then recombinantly expressed in Xenopus oocytes. These receptors were studied using simultaneous voltage-clamp and micro-fluorometry. We successfully labeled α1-R186C, β2-K298C, α1-N275C, γ2-S195C and β2-I205C with sulfhydryl-reactive rhodamine derivatives. We then monitored the fluorescence change induced by different agonists (GABA and β-alanine), a competitive antagonist (SR-95531) and an allosteric modulator (diazepam). Agonists and antagonists induced similar conformational changes in loop F of α1 subunit, whereas they evoked the different conformational changes in loop F of the β2 subunit (antagonist induced a 52%±9.8% smaller fluorescence change than GABA induced (n>4, p<0.01). In loop F of the γ2 subunit we observed fluorescence changes induced by GABA and diazepam (n>4). The M2-M3 linkers of both the α1 and β2 subunits produced different fluorescence changes with agonists and antagonist (n>4). From our preliminary study, α1-R186C and β2-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

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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 (1μM) 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 (1μM) had no effect on these synaptic burst responses (n=4). However, after inhibiting synaptic NMDARs with the use-dependent channel blocker MK-801 (10μM) 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-202

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

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BACKGROUND: Kainic acid (KA) is widely used in epileptogenesis study and nitric oxide (NO) is linked in KA-induced seizure. Hipppocampal 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-203

### GROUP I METABOTROPIC GLUTAMATE RECEPTORS TONICALLY REGULATE SYNAPTIC $GABA_A$ RECEPTOR FUNCTION IN MIDBRAIN NEURONS

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$GABA_A$ receptors mediate the principal form of fast synaptic inhibition in the brain. Although phosphorylation of $GABA_A$ 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 $GABA_A$ 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 $GABA_A$ 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-βS to the patch pipette (n=4) and mimicked/occluded by substituting physiological cations in the patch pipette for $Tris^+$ (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 $GABA_A$ 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-204

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

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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 (±SEM) mEPSC rate was 6.1 ± 0.9. In control conditions superfusion of Ang II (1μM) caused a significant decrease (34%) in mEPSC rate to 4.0 ± 0.8 (n=18, $P \leq 0.01$ paired t-test), without affecting the mEPSC amplitude. In 5 of these neurons addition of the AT1 receptor antagonist, Candesteran (1μM), 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

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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 β-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-206

### THE INTRA-CORTICAL ORIGIN OF ABSENCE-LIKE SEIZURES IN THE GAERS MODEL IS LOCATED IN THE SOMATOSENSORY S2 CORTEX

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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±s.e.m., 146±31μA vs 257±56μA, 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 inherent 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-207

### INHIBITION IN THE LATERAL VESTIBULAR NUCLEUS

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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 $GABA_A$, 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 μm). 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). $GABA_A$ergic and glycinergic mIPSCs were recorded in the presence of TTX (1 μm) and CNQX (10 μm) and their respective antagonists, strychnine (1 μm) and bicuculline (10 μm). Recordings from 45 neurons showed a differential inhibitory input to Deiters and interneurons. Deiters neurons received predominantly $GABA_A$ergic inhibitory input, of very high frequency (mean frequency = 13.25 Hz, n=7), while interneurons received both $GABA_A$ergic and glycinergic inputs. Preliminary results also show a rostrocaudal difference in the degree of $GABA_A$ergic and glycinergic input onto Deiters neurons.

## POS-MON-208

### EFFECTS OF SIMVASTATIN AND 6-HYDROXYDOPAMINE LESION ON HISTAMINERGIC H1 RECEPTOR BINDING IN RAT BRAINS

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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 [3H]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

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We have previously shown that noradrenaline (NA) activates $\alpha_1$-ARs in barrel cortex. We now show that $\alpha_1$-AR activation causes $Ca^{2+}$ release from presynaptic stores modulating transmitter release. **Purpose:** To characterise how $\alpha_1$-ARs cause $Ca^{2+}$ mobilisation from presynaptic stores. **Methods:** 300 µm 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±1°C in the presence of tetrodotoxin (1 µM) and gabazine (3 µM). $\alpha_1$-ARs were activated by NA (10 µM) and β-ARs were blocked by propranolol (PO; 1 µM). $IP_3$ receptors were blocked by 2-APB (16 µM). SERCA pump was blocked by cyclopiazonic acid (CPA; 20 µM) in conjunction with a brief $K^+$ depolarisation to deplete the $Ca^{2+}$ stores. Superfusion rate was 4 mL/min. **Results:** Co-application of PO (n=8) with NA showed a 30±5% increase in mEPSC frequency (from 39±5 to 51±5 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±4% (41±3 to 31±4 Hz) and the subsequent NA application did not increase mEPSC frequency. When stores were depleted (CPA application plus $K^+$ depolarisation; n=12), a significant drop of 17±2% in mEPSC frequency was observed (41±3 to 34±3 Hz), which was not reversed by NA application. **Conclusions:** Presynaptic $\alpha_1$-ARs and β-ARs are present in rat somatosensory cortex and signalling via $\alpha_1$-ARs causes $IP_3$ production leading to the activation of $Ca^{2+}$ release from stores.

## POS-MON-210

### INTERFERON-INDUCED FUNCTIONAL CHANGES IN 5-HT2C RECEPTORS AND KV1 CHANNELS IN MOUSE BRAIN

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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-HT2C 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 IFNα production. In hippocampal pyramidal neurons in brain slices from untreated control mice, activation of 5-HT2C 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-HT2C and Kv1 function were abolished in IFN-α/β receptor (IFNAR) knockout mice, confirming a causative role of type 1 IFNs. Our data suggest that functional modulation of 5-HT2C 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-211

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

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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-212

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

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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 µM, $n$=7) and 4-AP (200 µM, $n$=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±1.6 mV, $n$=6; SP: -25.7±1.6 mV, $n$=7; $p<0.001$; inactivation: SL: -85.9±1.1 mV, $n$=7; SP: -78.1±1.2 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

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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 µm 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

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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 ($sI_{AHP}$); however, it is controversial as to whether the channels underlying the $sI_{AHP}$ 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 ± 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 ± 3 %). Stimulation of β-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 $sI_{AHP}$ channels.

## POS-MON-215

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

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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

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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 9253µm of apical dendrite (11 neurons from 9 mice). After six hours, we found that 21.4% ± 5.2% (n=362) of spines had disappeared and 23.0% ± 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

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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 6C). 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±0.39 and -27.4±2.2mV (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±1.4 and -68±2.2mV (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-218

### POSTNATAL REFINEMENT OF SYNAPTIC DYNAMICS BETWEEN LAYER 5 PYRAMIDAL NEURONS IN RAT VISUAL CORTEX

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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-219

### A TWO-STEP PROCEDURE TO FIT SYNAPSE MODELS TO EXPERIMENTAL DATA

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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-220

### DENDRITIC SPINES PROMOTE SYNAPTIC EGALITARIANISM

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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)

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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 $GABA_A$ and $GABA_B$ 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 ± 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

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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 µm-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 ± 0.2 ($n$=5 pairs) times larger in SL cells, whereas stimulation of *assn* inputs elicited EPSCs that were 7.7 ± 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 ± 9.5 pA, $n$=29; SP: 19.4 ± 2.3 pA, $n$=20; $p<0.01$) but of similar size with *assn* stimulation (SL: 42.6 ± 4.9 pA, $n$=10; SP: 41.3 ± 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

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**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 µ-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 µ- and κ-opioid agonists, DAMGO and U69593 produced a reduction of evoked inhibitory postsynaptic currents (IPSCs) in PAG output neurons, while the δ-opioid agonist, deltorphin had no significant effect. Hence, both µ- and κ-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

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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)

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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-226

### USING PHOTORHODOPSINS TO PROBE NEURONAL CIRCUITRY

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**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 $8x10^7$ infectious units/ml. Animals stereotaxically 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-227

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

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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 µm 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-228

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

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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$^{NTR}$ TARGETED NON-VIRAL GENE DELIVERY IN WILD TYPE AND SOD1$^{G93A}$ PRIMARY MOTOR NEURON CULTURES

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**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$^{NTR}$ expressing motor neurons using primary embryonic motor neuron (PMN) cultures and mixed cultures (PMNm) from wild type and SOD1$^{G93A}$ transgenic mice. **Methods:** Monoclonal antibody to the neurotrophin receptor p75$^{NTR}$ (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$^{G93A}$ 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$^{G93A}$ PMN cultures. Reduced toxicity and stability of the immunoporter 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

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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 bification 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 MULTIFOCAL PUPILLOGRAPHIC RESPONSES

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**Purpose:** Multifocal pupillography perimetry 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$^2$ luminance, second experiment (n=22) at 150cd/m$^2$ 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 (-1.58µm, p=0.0001) but increased constriction for yellow stimuli (1.15µm, p=0.006). These results were verified in Experiment 2; white stimulus responses were suppressed prominently the inner two eccentricity rings: Ring1: -0.25dB; Ring2: -0.38dB; and Ring5 (-0.28dB) 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 seems 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

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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 tegmentel 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 ($p \leq 0.025$, 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

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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-234

### MAGNETIC RESONANCE SPECTROSCOPIC VARIATIONS BETWEEN HEMISPHERES

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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 Seimens' 1.5T whole body scanner at the John Hunter Hospital, Newcastle. Voxels (1cm3) 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-235

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

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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 36oC. 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-236

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

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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 You-tube. 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

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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 amygdalohippocamplal area, and the basomedial amygdaloid nucleus, anterior part (BMA; called BMMC 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

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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

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**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

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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 $Q_O$ SITE OF MITOCHONDRIAL COMPLEX III AS THE SOURCE OF INCREASED PRODUCTION OF SUPEROXIDE IN CARDIAC MYOCYTES AFTER TRANSIENT EXPOSURE TO HYDROGEN PEROXIDE

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Oxidative stress is a feature of cardiovascular disease. We have previously shown that exposure of adult guinea-pig ventricular myocytes to 30μM hydrogen peroxide ($H_2O_2$) for 5min results in increased mitochondrial superoxide production. This causes a 2-fold increase in protein synthesis, suggesting transient exposure to $H_2O_2$ 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 $Q_O$ ROS generation site and examined superoxide generation assessed as changes in dihydroethidium (DHE) fluorescence after exposing myocytes to 30μM $H_2O_2$ 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 $Q_O$ 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 $Q_i$ ROS generation site did not alter the DHE signal after exposure to 30μM $H_2O_2$. These data suggest the source of ROS production after transient exposure to $H_2O_2$ is the $Q_O$ 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-242

### AN IMPROVED OPEN CHANNEL STRUCTURE OF MSCL

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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-243

### $GABA_A$ RECEPTORS INCREASE THEIR CONDUCTANCE THROUGH NOVEL PROTEIN INTERACTIONS

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Native $GABA_A$ 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 $GABA_A$ receptor abrogates the potentiating effect of diazepam on both endogenous receptors and recombinant $GABA_A$ 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 $GABA_A$ 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-244

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

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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

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The electrotonic length constant (λ) 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 λ 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 μs) 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 λ is given that applies to conditions relevant to neuroprothetic stimulation. It predicts that the length constant depends on the duration of the stimulus pulse, but is largely independent of neural type. Estimates of λ range from 3 to 30 μm, 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?

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**INTRODUCTION:** Neurogenesis has been observed in the hippo-campus 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 gyral 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 progenitors 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 > 10μm 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

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**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 μm) 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 μM) reduced the EPSP amplitude at L5-L5 pairs to 59 ± 15% of control (n=8, $p<0.05$). In contrast at L2/3-L5 pairs AP5 reduced EPSPs to 79 ± 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

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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.