POSTER SESSION B

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Posters 029 - 082	Clinical Disorders & Injury of the Nervous System
Posters 083 - 115	Cognition, Learning & Behaviour
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POS-THU-001

NEUROPROTECTIVE EFFECT OF THE TOTAL FLAVANONES FROM IRIS TENUIFOLIA AGAINST OXIDATIVE STRESS-INDUCED CELL DEATH THROUGH THE ACTIVATION OF PHOSPHORYLATION OF MITOGEN-ACTIVATED PROTEIN KINASE

A. JALSRAI[1], T. NUMAKAWA[2], N. ADACHI[2], H. KUNUGI[2];

[1]Ctr. of Traditional Med., Ulaanbaatar, Mongolia; [2]Dept. of Mental Disorder Res., Natl. Inst. of Neurosci., Tokyo, Japan

Increasingly flavonoids have been observed to exert their cellular effects via the modulation of signaling pathways, such as the MAPK pathway. The flavanones possess neuroprotective effect against H2O2-mediated neurotoxicity, neurons were pre-treated with the flavanones for 24 h prior to the addition of hydrogen peroxide. The greatest degree of protection evident at 1.6 mM. We found that total flavanones induced the activation of both MAPK/ERK and AkT (a component of the PI3K pathway). The activation of both the MAPK and PI3K pathways indicate it's involving in protection action of total flavanones. The pattern of protection induced by total flavanones directly mirrored their potential to modulate the AkT and ERK1/2 kinase which followed a bell-shaped activation curve with time-course in response to the flavanones. Furthermore, the ability of total flavanones to inhibit hydrogen peroxide-induced cell death was significantly blocked by U0126 and LY294002 inhibitors that confirmed involvement of both MAPK and PI3K pathways in the protection effect of total flavanones from Iris tenuifolia.

POS-THU-002

ANATOMICAL CONNECTIVITY OF PUTATIVELY RHYTHMOGENIC NEURONES OF THE NUCLEUS RETROAMBIGUUS WITH THE CENTRAL PATTERN GENERATOR FOR BREATHING.

Jones SE, Dutschmann M, Stanic D.

Florey Instiute of Neuroscience and Mental Health

Purpose: Precise neuronal mechanisms of respiratory rhythm generation are rather equivocal. Precedence has been given to the pre-Bötzinger complex, labelled the 'rhythmogenic kernel' of respiration, while the contribution of other network areas remains unclear. We recently investigated a putative rhythmogenic area 1.5 mm caudal to obex, destruction of which abolished respiratory rhythm in the in-situ perfused brainstem-spinal cord preparation of rat (see Jones et al., Respir Physiol Neurobiol 2012). Histological analysis indicated that the putative rhythmogenic region is likely to be the nucleus retroambiguus (NRA) due to its identified location; caudal to the lateral reticular nucleus and containing a cell population extending from the pyramidal decussation into the upper half of spinal cord segment 1. Here, we investigated the possible involvement of the NRA in rhythm generation by mapping its neuronal connectivity in the rat. We aimed to determine whether the region is linked anatomically with other key areas of the respiratory pattern generator. Methods: Anterograde neuronal tracer, biotinylated dextran amine (BDA), was injected into the NRA. Respiratory areas in which labelled terminal fields were seen were injected with the retrograde tracer Fast Blue in subsequent experiments. Results: Anterograde tracing from the NRA revealed dense clusters of labelled nerve fibers and terminal boutons within important respiratory nuclei including the pre-Bötzinger complex, parafacial respiratory group and Kölliker Fuse nucleus (KF). Accordingly, injection of Fast Blue into the pre-Bötzinger complex and KF both showed clusters of retrogradely labelled cell bodies in the NRA. Conclusion: We have confirmed anatomical connectivity between the rhythmogenic cluster within the NRA and designated key areas of the respiratory pattern generator. This likely provides ascending drive to the respiratory central pattern generator, which is crucial to the production and/or maintenance of an eupneic respiratory rhythm.

POS-THU-003

OREXINERGIC ACTIVATION OF MEDULLARY PREMOTOR NEURONS MEDIATES THE ADRENAL SYMPATHOEXCITATION TO HYPOTHALAMIC GLUCOPRIVATION.

Willian S. Korim[1], Lama Bou-Farah[2], Simon McMullan[2] and Anthony J.M. Verberne[1]

1. University of Melbourne
Clinical Pharmacology and Therapeutics Unit Department of Medicine
Austin Health, Heidelberg, VIC 3084 Australia
2. Macquarie University
Australian School of Advanced Medicine Sydney, Australia

Glucoprivation activates neurons in the perifornical hypothalamus (PeH) and in the rostral ventrolateral medulla (RVLM), which results in release of adrenaline. The current study aimed to establish *in vivo* (i) whether neuroglucoprivation in the PeH or in the RVLM elicits adrenaline release; and (ii) whether direct activation of RVLM neurons by glucoprivation or the orexinergic drive to the RVLM mediates the release of adrenaline. Neuroglucoprivation in the PeH or RVLM was elicited by microinjections of 2-deoxy-D-glucose or 5-thio-D-glucose in anesthetized, euglycemic, rats. We found that inhibition of neurons in the PeH abolished the increase in adrenal sympathetic nerve activity (ASNA) to systemic glucoprivation. Secondly, glucoprivation of neurons in the PeH elicited an increase in ASNA. Thirdly, *in vivo* or *in vitro* glucoprivation did not affect the activity of RVLM adrenal premotor neurons. Finally, blockade of orexin receptors in the RVLM abolished the increase in ASNA to neuroglucoprivation in the PeH. The evoked changes in ASNA were directly correlated to levels of plasma metanephrine, but not to normetanephrine. These findings suggest that glucoprivation in the PeH activates orexin inputs to the RVLM, capable of activating adrenal medullary presympathetic neurons in the RVLM.

POS-THU-004

ABLATION OF AREA MT IN EARLY LIFE HAS SIGNIFICANT IMPACT ON THE REMAINING ANATOMY OF THE VISUAL SYSTEM, INCLUDING V1

William C Kwan, Claire E Warner, James A Bourne
Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria, Australia.

The visual cortex of the primate has been defined to contain two serial streams, which are responsible for discerning specific spatial or form aspects of visual perception. The dorsal stream processes spatial features of the visual scene, and develops more precociously than the ventral stream. The middle temporal area (area MT) is a major component of the dorsal stream and has previously demonstrated to be a 'primary like' area, recipient of direct afferent input from the visual thalamus (LGN and pulvinar). Therefore, to demonstrate that area MT plays an important role in the establishment of the dorsal stream, we examined the effect of ablating the area in early life. In the marmoset monkey (*Callithrix Jacchus*), we unilaterally ablated area MT in neonates (PD14; n=2) and adults (2.7 years; n=2), and allowed them to recover for a minimum of 12 months before further examination. Similar to marmosets which undergo a unilateral ablation of V1, we observe retrograde transneuronal degeneration (RTD) in the foveal retina, and layer K3 in the LGN. However with MT ablations we also observed RTD in the pulvinar nucleus. These data suggest that pathways providing input to area MT which bypass V1 are significantly effected following a lesion of area MT in early life, which likely has significant impact on the remaining visual cortex, especially areas associated with the dorsal stream.

POS-THU-005

VALIDATING AN ANIMAL MODEL FOR OLANZAPINE-INDUCED OBESITY: RESPONSES TO WITHDRAWAL AND REINTRODUCTION OF OLANZAPINE

Lian J [1,2], Huang X-F[2], Pai N [2], Deng C[1,2]

1. Antipsychotic Research Laboratory, Illawarra Health and Medical research Institute, University of Wollongong, Wollongong, Australia
2. Centre for Translational Neuroscience, School of Medicine, University of Wollongong, Wollongong, Australia

Olanzapine, as the first line antipsychotic drug, is widely prescribed to treat schizophrenia and other mental disorders. However it is associated with troublesome weight gain/obesity side-effects. This study aimed to further validate an animal model for olanzapine-induced weight gain. Methods: Female Sprague-Dawley rats were administered under 3 phases: Phase 1, rats were treated with olanzapine (1 mg/kg, t.i.d.) or vehicle for 23 days; Phase 2, from Day 23, olanzapine was withdrawn for 19 days; Phase 3, olanzapine (1 mg/kg, t.i.d.) was re-introduced for 5 weeks treatment. Results: Olanzapine significantly increased food intake and body weight gain compared to vehicle through the Phase 1 (all $p<0.001$). However, followed olanzapine withdrawal in the Phase 2, weight of the olanzapine-treated rats was gradually declining, and then reduced to a level similar to controls after 12 days of drug withdrawal ($p>0.05$). Similar to the changes in weight loss, olanzapine withdrawal also leaded gradually decrease in food intake and feeding efficacy to the level similar to control ($p>0.05$). In Phase 3, resumed olanzapine treatment caused significant increase of food intake, feeding efficacy and weight gain again. The significant weight gain was maintained throughout the olanzapine treatment period. Furthermore, olanzapine-treated rats significantly increased inguinal fat ($p<0.001$), and periovary, mesentery fat ($p<0.05$) compared to controls. The hypothalamic POMC protein expression was downregulated by resumed olanzapine treatment compared to controls ($p<0.05$). Conclusion: This study provided an animal model well mimicking body weight changes caused by olanzapine in drug naïve and re-administrated chronic treatment patients.

POS-THU-006

THE EFFECT OF BOTULINUM TOXIN TYPE A ON NEUROTRANSMISSION IN PORCINE BLADDER

Bahadory F.[1], Burcher E.[1], Mansfield K. J.[2], Moore K. H.[3], Liu L.[1]

[1.]School of Medical Sciences, University of New South Wales, Sydney, NSW; [2.]Graduate School of Medicine, University of Wollongong, Wollongong, NSW; [3.]Dept of Urogynaecology, St George Hospital, University of New South Wales, Sydney, NSW

It is well established that onabotulinum toxin A (Botox) inhibits cholinergic neurotransmission through the cleavage of neuronal synaptosomal-associated protein-25 (SNAP-25). However, its effect on the release of other neurotransmitters is much less studied. Purpose: To examine the effect of Botox on electrical field stimulation (EFS)-induced release of ACh, ATP and substance P (SP), and to investigate the expression of SNAP-25 in porcine bladder. Methods: Bladder dome segments from female pigs were injected with Botox (1, 5 & 20U/ml, 12h incubation), longitudinal strips of mucosa and detrusor were stimulated at 2-20Hz, 0.1ms, 100V. Bath fluid was collected for measuring neurotransmitter release. Expression of SNAP-25 was investigated by real-time PCR and immunofluorescence. Results: EFS produced a frequency-dependent contractile response in both mucosa and detrusor strips, which was significantly inhibited or abolished by Botox. EFS-induced release of ACh was similar in detrusor and mucosa. In contrast, more ATP and SP were released from mucosa than detrusor. Botox significantly decreased (5U/ml) or abolished (20U/ml) the release of SP and ACh. ATP release was significantly inhibited by Botox at all concentrations. SNAP-25 mRNA was highly expressed in both mucosa and detrusor. There was dense SNAP-25 immunostaining on nerve endings, and also on urothelial cells and myofibroblast-like cells in mucosa. This density was greatly reduced with Botox treatment. Conclusion: The reduction in release of ACh, ATP and SP, as well as SNAP-25 positive cells in mucosa and detrusor, suggests that Botox acts in both regions, and that Botox may affects afferent in addition to efferent signalling.

POS-THU-007

PANNEXIN-1, AN ATP RELEASE CHANNEL, IS DOWNREGULATED IN THE COLONIC MUCOSA OF PATIENTS WITH SLOW TRANSIT CONSTIPATION

Markus I.[1], Diezmos E.F.[1], Sandow S.L.[1], Bertrand P.P.[2], and Liu L.[1]

[1.]School of Medical Sciences, University of New South Wales, Sydney, NSW; [2.]School of Medical Sciences, RMIT University, Bundoora, VIC

Slow transit constipation (STC) is a colonic dysmotility, characterised by the inability to defecate more than once every 2-3 weeks. STC predominantly occurs in young to middle-age females. To date, no efficacious treatment is available due to the unknown mechanisms of this disorder. Pannexins (Panxs) are a novel group of membrane-spanning protein channels, composed of Panxs1-3, among which Panx1 represents the best studied isoform. It is ubiquitously expressed and mediates ATP release. Purpose. This work examined whether abnormal expression of Panxs occur in the colon of STC patients. Methods. Sigmoid colon segments were obtained from age-matched female patients (aged 23-69 years) undergoing resection for STC (*n*=10), or for carcinoma (normal colon, 30-68 years, *n*=12). Colon specimens were separated into mucosa and muscle layers. Each layer was analysed for Panx gene expression by real-time PCR. Immunohistochemistry was conducted with intact tissues to evaluate the localisation of Panx1 and Panx2 proteins. Results. Genes encoding Panx1 and Panx2, but not Panx3 were expressed in both muscle and mucosa layers of the colon. Panx1 and Panx2 immunoreactivity (IR) was detected on myenteric and submucosal ganglia. Moreover, strong Panx1-IR staining was seen on blood vessel endothelium and erythrocytes, and moderate staining on epithelial cells, goblet cells, and smooth muscle. Panx2-IR was present on the smooth muscle of blood vessels. In STC mucosa, there was a 2-fold down-regulation of Panx1 ($P < 0.01$, Mann Whitney test) compared to control. Panx2 expression remained unchanged in STC mucosa. Neither Panx1 nor Panx2 showed an altered expression in STC muscle. Conclusion. Widespread Panx1 and Panx2 expression in the colon suggests multiple roles for mediating gut functions. Altered Panx1 level in STC mucosa may result in faulty ATP signalling, which affects sensory impulses of mucosal origin, thus impairing the colonic peristaltic reflex and/or the colonic migrating motor complex in STC patients.

POS-THU-008

DAMAGE TO SUBMUCOSAL NEURONS AS A RESULT OF TREATMENT WITH ANTI-CANCER AGENT IRINOTECAN

McQuade R[1] Nurgali K[1]

[1]College of Health and Biomedicine, Victoria University, Australia

Purpose: Anti-cancer agent Irinotecan is a commonly used chemotherapeutic in patients diagnosed with colorectal cancer. Common side-effects of this therapy include nausea, vomiting, constipation and diarrhoea that can persist up to 10 years post-treatment. Secretomotor neurons responsible for secretion throughout the gastrointestinal tract are located within the submucosal plexus. We studied the effects of Irinotecan on these neurons to understand mechanisms underlying gastrointestinal side-effects. Methods: Irinotecan (30 mg/kg/d) was administered *in vivo* to Balb/c mice intraperitoneally three times a week. Wholemount and cryostat preparations of proximal ileum, jejunum and colon were examined immunohistochemically in Irinotecan and sham-treated mice at days 3, 7 and 14 following injections. Submucosal neurons were labelled using pan-neuronal marker PGP 9.5. Subpopulations of secretomotor neurons were labelled using antibodies to Vasoactive Intestinal Peptide (VIP), Neuropeptide Y (NPY) and Choline Acetyl Transferase (ChAT). Structural damage was assessed histologically. Results: Significant decrease in total number of submucosal neurons ($p<0.05$) as well as changes in proportion of VIP, NPY and ChAT-immunoreactive neurons across all time points (n=6/group) were found in Irinotecan-treated mice compared to sham-treated. Damage to intestinal mucosa, morphological changes in neuronal cell bodies and decrease in average size of ganglia were seen following Irinotecan injection.
Conclusion: This study is the first to examine the effects of Irinotecan on secretomotor neurons. Results demonstrated that repeated exposure to Irinotecan causes significant neuronal loss and decreases in protein expression, ganglia size and changes to neuronal cell body morphology, which may be contribute to functional changes within the gut.

(248 words)

POS-THU-009

EVIDENCE FOR BRAINSTEM INFLAMMATION FOLLOWING RESPIRATORY PNEUMOVIRUS INFECTION IN MICE.

Narula M1, Yang S1, McGovern AE1, Eacharath J1, Phipps S1, Mazzone SB1.

1. School of Biomedical Sciences, University of Queensland, St Lucia QLD.

Respiratory tract viral infections acutely induce airway neural protective reflexes and in some individuals lead to chronic reflex hypersensitivities which are difficult to treat. Although respiratory viruses can alter the physiology and phenotype of airway sensory neurons, little is known about the possible central neural responses that might contribute. We investigated whether respiratory tract infection with pneumovirus (PVM) leads to inflammation in brainstem nuclei regulating airway functions. Postnatal day 7 C57BL/6 wildtype (WT) mice (n=20) or mice deficient in the viral recognition receptor RAGE (RAGE-/-, n=20) were exposed to 10pfu (intranasally) of PVM or vehicle and their brainstems harvested at 3, 7 or 10 days post-inoculation for immunohistochemical analysis. Astrocytes (GFAP+), microglia (IBA1+), proliferating cells (PCNA+), activated neurons (c-Fos+) and injured neurons (c-Jun+) were quantitatively assessed in sensory and motor nuclei. In WT mice, PVM infection induced a significant ($P<0.05$) increase (peaking at 7-10 days post-infection) in GFAP, IBA1 and PCNA expression in the nucleus of the solitary tract, the caudal spinal trigeminal nucleus and/ or the nucleus ambiguus and this was associated with significantly ($P<0.05$) increased number of c-Fos and c-Jun expressing cells in these sensory and motor nuclei. The magnitude of the changes observed in RAGE-/- mice was more pronounced and occurred earlier (3-7 days post-infection) consistent with the significantly increased severity of PVM infection in the airways of these animals. Respiratory viral infections promote injury and inflammation within the brainstem nuclei regulating airway reflexes. These effects may contribute to airway hyperreflexia in individuals with infectious respiratory diseases. (250 words).

POS-THU-010

INCREASE IN VOIDING AT EARLY STAGE OF BLADDER OBSTRUCTION IS UNLIKELY TO BE DUE TO CHANGES IN CONTRACTILE OR EFFERENT NEURAL ACTIVITY

Nicholas S, Brookes SJH, Spencer NJ, Costa M and Zagorodnyuk VP

Human Physiology and Centre for Neuroscience, FMST, School of Medicine, Flinders University, SA, Australia

Bladder overactivity could be due to changes in the activity of smooth muscle/interstitial cells, efferents and/or afferents involved in micturition reflex pathways. Our aim was to determine whether enhanced voiding in bladder overactivity correlates with increased bladder contractility in vitro. Partial bladder outlet obstruction in guinea pigs was used to produce bladder overactivity. Spontaneous voiding of sham-operated and four weeks obstructed guinea pigs was measured in metabolic cages. Distension (2ml)- and electrical field stimulation (EFS)-induced contractile activity were recorded, in vitro, via a pressure transducer. Voiding frequency (control 0.27±0.05 per 1 hr, n=15) was significantly ($P<0.05$) increased by the 1st (1.01±0.18), 2nd (1.17±0.17), 3rd (1.75±0.5) and 4th (1.37±0.32) weeks after obstruction. In contrast, voiding frequency of the sham-operated group (n=7) was increased only at the 1st (0.75.1±0.1, $P<0.05$) week and then returned to control levels at 2nd (0.39±0.08), 3rd (0.39±0.1) and 4th (0.3±0.08) weeks. Bladders from the obstructed group were heavier than the sham-operated group ($P<0.001$). There was no difference in frequency or amplitude of spontaneous and distension-induced contractile activity between obstructed (n=13) and sham-operated groups (n=7). Obstructed bladders had lower compliance than the sham group. When normalised to high KCl, responses of obstructed bladders to EFS (n=6) were significantly lower than sham (n=4, $P<0.05$), without differences in the relative contribution of either cholinergic or purinergic components. The data suggest that significant increase in conscious voiding of obstructed guinea pigs is unlikely to be due to changes in contractile or efferent neural activity. (245 words).

POS-THU-011

UNDERSTANDING MECHANISMS OF RECTAL PROLAPSE IN THE MOUSE MODEL OF SPONTANEOUS CHRONIC COLITIS: DAMAGE TO THE MUSCLES AND NERVES

Authors: A.A. Rahman[1], S Randall-Demllo[2], S. Carbone[1], V Jovanovska[1], R Eri[2], K Nurgali[1]
[1]Victoria University, Melbourne, Australia, [2]University of Tasmania, Launceston, Australia

Patients with symptomatic rectal prolapse often suffer from constipation, incomplete rectal evacuation, excessive straining at stool, fecal incontinence, anal pain and rectal ulcers. The aetiology underlying rectal prolapse is unclear and have been linked to advanced age, chronic constipation/diarrhoea, increased abdominal pressure, and prior hysterectomy. Enteric nervous system (ENS) is the key regulator of intestinal motility and abnormalities of the ENS are associated with gastrointestinal motility disorders. We hypothesise that alterations of the ENS and extrinsic innervation of the colorectum play a critical role in the pathogenesis of rectal prolapse. *Winnie* mice with spontaneous chronic colitis (C57/BL6 background) often develop rectal prolapses. The aim of this study was to investigate the alterations in sensory, sympathetic and parasympathetic innervation of the colorectum in *Winnie* mice.
Results: The density of calcitonin gene-related peptide (CGRP)-immunoreactive (IR) sensory afferent fibres is increased, but the density of parasympathetic cholinergic (VAChT-IR) fibres and sympathetic adrenergic fibres (TH-IR) are significantly reduced in *Winnie* mice with prolapse compared to controls. The total number of myenteric neurons (PGP9.5-IR) and nNOS-IR neurons per ganglion is significantly reduced in rectal prolapse compared to controls (PGP9.5: 21.3±1.9 vs 26.4±0.9; nNOS: 9.9±0.7 vs 13.7±1.1; $P<0.05$, n=3/group).
These changes in the ENS correlated with increase in propulsive activity and speed of colon contractions in *Winnie* compared to controls. These findings provide evidence that *Winnie* mice with rectal prolapse have significant changes in sensory, sympathetic and parasympathetic innervation of the colorectum correlating with changes in neurally- controlled gut functions and symptoms observed in these mice.

POS-THU-012

DIFFERENTIAL EFFECTS OF ARIPIPRAZOLE AND HALOPERIDOL ON THE NIGROSTRIATAL AND MESOCORTICAL DOPAMINERGIC PATHWAYS IN MALE RATS

Bo Pan, Xu-feng Huang, Chao Deng,
School of Medicine, and Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, Australia

Aripiprazole is a new antipsychotic drug with a high affinity for dopamine D_2 receptors but has low extrapyramidal side-effects. Although aripiprazole is considered to be a D_2 partial agonist, emerging evidence suggests that the unique activity of aripiprazole may be due to its functional selectivity on D_2 receptors. Aim: the present study aimed to investigate the effects of aripiprazole on two dopamine D_2-coupled signalling pathways – G-protein dependent cAMP-PKA pathway and G-protein independent β-arrestin2-Akt-GSK3 pathway. As a comparison, the effects of haloperidol (a D_2 antagonist) and bifeprunox (a D_2 partial agonist) were also examined. Methods: Male Sprague Dawley rats were injected once with aripiprazole (0.75mg/kg, i.p.), haloperidol (0.8mg/kg, i.p.), bifeprunox (0.1mg/kg, i.p.) or saline (n=6/group). Protein levels of PKA regulatory (PKA-r) and catalytic subunit (PKA-c), Akt, phosph-Akt, GSK3 and phosph-GSK3 in the prefrontal cortex (PFC), ventral tegmental area (VTA), caudate putamen (CPu) and substantia nigra (SN) were measured. Results: PKA-r level was increased by all three drugs in VTA, by aripiprazole and haloperidol in CPu and by bifeprunox in SN. PKA-c level was decreased by aripiprazole in PFC and by all three drugs in CPu; however, aripiprazole and bifeprunox promoted it in VTA. Both aripiprazole and bifeprunox enhanced GSK3 activity in all four brain regions; however, haloperidol reduced GSK3 level in PFC, VTA and CPu. Additionally, Akt level was decreased by aripiprazole in PFC and VTA, and by haloperidol in VTA and CPu. Conclusion: These results revealed that aripiprazole and haloperidol affected dopamine D_2-coupled signalling pathways differently in various brain regions.

POS-THU-013

THE REGENERATIVE AND PLASTIC BEHAVIOR OF PELVIC AUTONOMIC AND SENSORY NEURONS FOLLOWING AXOTOMY

Payne SC, Keast JR.
Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Australia.

Autonomic neurons of the pelvic ganglia (PG) innervate urogenital organs. Damage to their axons and associated sensory nerves causes loss of bladder control, a debilitating complication of pelvic surgery. The capacity of pelvic sensory and motor neurons to reinnervate the bladder, either by regeneration of damaged axons or compensatory growth of spared axons, is poorly understood. Experiments were conducted on adult male Wistar rats (n=4/group), with surgery performed under isoflurane anaesthesia. The bladder was denervated unilaterally (accessory nerve transection) and 4 weeks later retrograde tracer applied to the bladder to identify connections; different tracers were used on each side to determine the origin of new connections. Dorsal root ganglia (DRGs) and PGs were dissected 1 week later. Using immunohistochemistry, neuron phenotypes were identified, in addition to the regenerative markers, activating transcription-factor 3 (ATF-3) and c-Jun. Ipsilateral to injury, the number of dye labeled sensory neurons was similar to the contralateral side, indicating regeneration; significantly more of the sensory neurons in ipsilateral DRGs were peptidergic, and over 60% innervated both sides of the bladder. Little expression of ATF-3 or c-Jun was detected. Very few dye labeled motor neurons were present in ipsilateral PG, suggesting limited regeneration. Four weeks after injury, many neurons were labelled in contralateral PG and DRG, indicating growth of axon collaterals to the denervated area. Neuronal death was not detected in any ganglia. Taken together, axons of pelvic motor neurons have a limited capacity to regenerate compared to pelvic sensory neurons and pose a target for potential therapies.

(250)

POS-THU-014

BAROREFLEX CONTROL OF SPLANCHNIC, RENAL AND LUMBAR SYMPATHETIC NERVE ACTIVITY IS DIFFERENTIALLY REGULATED BY ANGIOTENSIN II IN CHRONIC KIDNEY DISEASE

Yimin Yao[1], Cara M Hildreth[1], Melissa M Farnham[1,2], Jacqueline K Phillips[1]

(1) Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia

(2) Heart Research Institute, University of Sydney, Newtown, Sydney NSW Australia

Our aim was to determine if there is divergent regulation of baroreflex control of splanchnic (sSNA) lumbar (lSNA) and renal (rSNA) sympathetic nerve activity by the renin angiotensin system (RAS) in chronic kidney disease (CKD). Using 12-week male Lewis Polycystic Kidney (LPK) or Lewis rats (*n* =8-10) under urethane anaesthesia, arterial pressure (AP), heart rate (HR), rSNA, lSNA and/or sSNA were recorded. Baroreflex function was studied with phenylephrine or sodium nitroprusside (i.v.). In a subset of animals (n=4-6) the RAS inhibitor losartan (3 mg/kg i.v.) was then administered. Baseline values of AP (82 ± 2 *vs* 93 ± 4mmHg), rSNA (3.1 ± 0.6 *vs* 8.5 ± 1.6µV), lSNA (2.3 ± 0.4 *vs* 6.9 ± 1.6µV) and sSNA (3.7 ± 0.7 *vs* 6.6 ± 1.2µV) were significantly higher in the LPK ($p<0.05$). Baroreflex functional curves were all right shifted (MAP_{50}) in the LPK and while the gain and maximal range for rSNA and sSNA were lower in the LPK ($p < 0.05$), these parameters were not different for lSNA. Losartan reduced AP (36 ± 3 vs. 16 ± 4mmHg, Lewis *vs.* LPK $p<0.01$) and left shifted baroreflex function curves for rSNA and lSNA in Lewis, and all nerve beds in the LPK. There was a significant improvement in the gain for sSNA in LPK rats only (-0.7 ± 0.04 *vs* - 1.2 ± 0.1, $p < 0.05$). This data indicates differential central processing of baroreflex control of SNA to specific target regions with selective modulation of sSNA by angiotensin II in CKD.

POS-THU-015

BLOCKADE OF ACID SENSING ION CHANNELS ALTERS MOTILITY OF GUINEA PIG PROXIMAL COLON

Polglaze KE, Bertrand PP
School of Medical Sciences, RMIT University, Bundoora, Australia

Previously we have shown that blockade of mechanosensitive acid sensing ion channels (ASICs) reduces 5-HT release from guinea pig ileum and colon, and reduces peristalsis in ileum. In this study our aim was to determine the effect of ASIC blockers amiloride or benzamil on motility of the proximal colon.

Short (5cm) segments of proximal colon from guinea pigs of either sex (amiloride: 497±94g, n=7; benzamil: 647±137g, n=7) were placed into an organ bath with warm, oxygenated physiological saline and cannulated at either end. Peristaltic threshold was defined as the pressure generated by an orally connected reservoir at which four successive propulsive contractions were observed. Data on time course were collected via a pressure transducer connected to the anal end. Percentages were expressed as of control and significance was set at $P<0.05$.

No change in threshold was observed in response to either drug (amiloride (300μM): 119±14%, benzamil (100μM): 97±19%). The proximal colon showed an unpredictable variety in pressure wave time course. Data were binned according to whether the pressure waves had a peak (amiloride n=4, benzamil n=3) or plateau (amiloride n=3, benzamil n=4), and only paired behaviours were analysed. The interval between contractions increased significantly in amiloride (peak - 162±15%) and benzamil (plateau - 146±25%). An increase in contraction duration was seen in amiloride (peak - 180±9%), benzamil (peak - 125±10%) and benzamil (plateau - 147±2%).

These data agree with increased time course of peristaltic contractions seen in ileum, suggesting a role for ASICs in modulation of peristalsis in proximal colon.

(250 words)

POS-THU-016

SEGMENTAL ORIGINS OF CARDIAC SYMPATHETIC NERVE ACTIVITY IN RATS.

Pracejus N, McAllen RM

The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, VIC 3010, Australia

Although information exists for other species, the spinal segments that give rise to cardiac sympathetic nerve activity in rats are unknown. To answer this question, we systematically disconnected segmental inputs whilst recording postganglionic cardiac sympathetic nerve activity in 6 artificially ventilated, urethane-anaesthetized rats (1.4 g/kg, i.v.). In preparatory surgery under 2% isofluorane anaesthesia, the upper thoracic sympathetic chain was exposed on the animal's left, after removal of the heads of ribs 2-4. The cardiac sympathetic nerve or nerves were identified exiting the stellate ganglion en route for the heart, and cut distally. The T1, T2, and T3 white rami were identified. Marking silk ties were threaded under these nerves as well as under the sympathetic trunk below T3. These preganglionic inputs were then successively cut whilst activity was recorded differentially from the cardiac sympathetic nerve under liquid paraffin. Cardiac sympathetic nerve activity was quantified from the rectified, smoothed signal after subtraction of noise. The deficit caused by each nerve section was then calculated as a percentage. The mean contributions from each segment were T1:12 ± 7.6%; T2: 25 ± 6.91%; T3: 28 ± 12.12%; T4 and below: 35 ± 11.6%. However, their relative contributions varied substantially between individual animals: in one case, 77% of activity originated below T3. We conclude that cardiac sympathetic activity in rats originates from more than one segment; T1 contributes little, and the major input is from T3 or below. (239 words).

POS-THU-017

IS REDUCTION OF FATTY ACID OXIDATION A STIMULUS FOR FOOD INTAKE?

Ritter R[1], Li A and Wang Q[1]

[1]Washington State University, Pullman WA, 99164-7620 USA

Mercaptoacetate (MA) reduces fatty acid oxidation by inhibiting acyl coA dehydrogenases. MA's metabolic action has been assumed to account for its ability to stimulate feeding, leading to the concept of a "lipoprivic" control of food intake. However, we recently identified a "nonmetabolic" action of MA that may contribute to MA-induced feeding. Using multiple *in vivo* and *in vitro* approaches, we found that MA blocks effects of long and medium chain fatty acids (FAs) at membrane G-protein coupled receptors 40 and 120 (GPR40 and GPR120), receptors that mediate effects of FAs on functions including fat taste and stimulation of gut peptides and insulin secretion. We found that MA blocks the effect of FAs and the GPR40 agonist, GW9508, on calcium influx in cultured nodose neurons, some of which express GPR40. MA also blocks FA induced calcium influx in STC-1 cells, GLP-1 secretion from STC-1 cells and *in vivo* secretion triggered by olive oil gavage. In addition, MA produced profound *in vivo* and *in vitro* blockade of insulin secretion and elevation of blood glucose. Finally, GPR40 KO mice do not increase food intake in response to MA. These findings reveal non-metabolic actions of MA on GPR40 and GPR120 that may contribute to its orexigenic effects. The interaction of MA with these receptors, known to control secretion of gut hormones that influence food intake in part or entirely by actions on the vagus nerve, is consistent with previous results showing that MA's feeding effects are vagally-dependent. NIH Grants DK040498 and DK081546 (248 words)

POS-THU-018

A NEUROPHYSIOLOGICAL STUDY OF THE MEDULLARY SYMPATHETIC PATHWAY TO THE ADRENAL GLAND

A. Sabetghadam, W. Korim. and A.J.M. Verberne

Neurons in the rostral ventrolateral medulla (RVLM) control adrenaline secretion from the adrenal gland and participate in glucose homeostasis. Here, we examined sympathetic evoked responses recorded from the adrenal sympathetic nerve in response to intermittent stimulation of the RVLM. All experiments were performed using isoflurane/urethane-anaesthetised, artificially-ventilated, paralysed, male Sprague-Dawley rats. The RVLM was first identified by extracellular single unit recording combined with field potential mapping of the facial nucleus. We compared the latencies of the evoked adrenal sympathetic nerve (ASNA) response to the lumbar sympathetic nerve (LSNA) response upon electrical stimulation of RVLM (0.5 Hz, 1 ms pulse width, 50-300 µA) after conversion to urethane (1.4 g/kg, i.v.). RVLM stimulation elicited an evoked ASNA response that depended on whether the recording was primarily pre- or post-ganglionic activity as judged by the effect of the ganglion blocker hexamethonium (Hex; 20-40 mg/kg, i.v.). Pre-ganglionic ASNA consisted of an early peak (latency - 79 $\pm$ 9 ms, n=4) and a late peak (latency - 149 $\pm$ 6 ms). Lumbar sympathetic nerve activity exhibited a prominent early peak (latency - 87 $\pm$ 7 ms; n = 5) that was eliminated by Hex. Pre-ganglionic ASNA was reduced by 62 % after intrathecal kynurenate (0.5 µmole/10 µl; $P<0.01$, late peak, n=3). The early peak may represent sympathetic vasomotor activity in both the adrenal and lumbar sympathetic nerves. However, the late sympathetic response noted in the adrenal nerve is mediated by a glutamatergic input and may be involved in the control of catecholamine secretion from adrenal chromaffin cells.

POS-THU-019

SKIN TO NEURONS: DERIVATION OF NEURONS FROM ADULT HUMAN SKIN NEURAL PRECURSOR CELLS (SKPS)

Authors: Ariel Seaton-Zozula1, Gilles J. Guillemin1, Trevor Lewis2, Andreas Loefler3, Michael Valenzuela4

Institutes:

1. The Australian School of Advanced Medical Sciences, Macquarie University, Macquarie Park NSW
2. School of Medical Sciences, The University of New South Wales, Sydney 2052
3. Prince of Wales Hospital,and Prince of Wales Private Hospital, Randwick NSW 2031
4. University of Sydney, The Brain and Mind Institute, Camperdown, NSW, 2051

Throughout life, our tissue and organs maintain a state of homeostasis and undergo repair due to the resident stem cells. During the neurodegenerative process, it is thought that the adult stem cells pool is in a sense, “used up,” due to either a high turnover of cells or the inability to convert resident stem cells into the required neural cell type. In this study, we characterize the ability of skin derived neural precursor cells (SKPs) as a source of autologous cells for the purpose of deriving neurons for future application.

Skin samples were obtained from patients undergoing orthopedic hip replacement surgery. The skin samples were processed, and stem cells isolated. SKPs were exposed to various factors, including a combination, Brain-derived neuronal factor, and Noggin during the differentiation process.

Preliminary data show that isolated SKPs are made up of a heterogeneous population, consisting of neural stem cells with markers such as Nestin, Pax6, βIntegrins, and Sox2, as well as embryonic markers, Oct-4 and SSEA4. Under the above conditions, SKPs are able to differentiate towards the neuronal lineage with a high degree of co-expressing β III Tubulin and Microtubule Associated Protein 2 (MAP-2). Most importantly, these differentiated SKPs begin to show neuronal function as early as day 12, demonstrated by the ability to generate action potentials accompanied by inward sodium channel activity.

Altogether this SKP-based system demonstrates the potential to use a less invasive avenue to derive patient -specific neural precursor and neuronal cells. In addition, this also provides an in vitro platform to model human neurodegenerative diseases.

POS-THU-020

INSULIN INDUCED HYPOGLYCAEMIA INCREASES ADRENALINE SECRETION AND TYROSINE HYDROXYLASE PHOSPHORYLATION IN RAT BRAIN AND ADRENAL GLAND

Senthilkumaran M, Johnson M, Bobrovskaya L

University of South Australia, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, Adelaide, Australia

In this study we aimed to determine the time course of adrenaline secretion and tyrosine hydroxylase (TH) phosphorylation in rat brain and adrenal gland in response to single insulin-induced hypoglycaemia. Overnight fasted rats received a single intraperitoneal injection of insulin (10U/kg) or saline and then were euthanised 30 min, 60 min or 90 min after injection (n=5-6 per group). Cardiac blood was collected for analysis of glucose and adrenaline. Adrenal glands and catecholaminergic brain regions were rapidly removed and TH phosphorylation at Ser40, Ser31, Ser19 and TH protein were analyzed by western blotting. Blood glucose levels were 6-8 mmol/L in control animals versus 2 mmol/L in insulin-treated animals between 30 and 90 min. Plasma adrenaline (measured by ELISA) was significantly increased at 30 min (20 fold; $p<0.05$), 60 min (30 fold; $p<0.001$) and 90 min (13 fold; $p<0.001$) relative to control. pSer31TH was significantly increased in the adrenal gland at all time points (4-5 fold; $p<0.01$), in C1 neurons at 30 and 60 min (2 fold; $p<0.05$), in locus coeruleus and striatum at 60 min (2 fold; $p<0.05$) and in substantia nigra at 60 at 90 min (2 fold; $p<0.01$). pSer19TH was significantly increased in substantia nigra and striatum at 60 and 90 min (1.3-2 fold; $p<0.05$). We provide evidence for the first time that TH phosphorylation in rat adrenals and brain is modulated in response to insulin-induced hypoglycaemia. The increased pSer31TH may be a mechanism to increase TH activation in catecholamine-producing cells in response to hypoglycaemia *in vivo.* (249 words).

POS-THU-021

MECHANISMS UNDERLYING HEXAMETHONIUM AND ATROPINE RESISTANT PERISTALSIS IN THE GUINEA-PIG DISTAL COLON

Sia TC[1,2], Brookes SJ[1], Dinning PG[2, 3], Wattchow DA[2] & Spencer NJ[1] *

[1]Department of Physiology, Flinders University, SA, Australia; [2]Department of Surgery, Flinders Medical Centre, SA, Australia; [3]Department of Gastroenterology, Flinders Medical Centre, SA, Australia.

Background: We recently described hexamethonium-resistant peristalsis in the isolated guinea-pig distal colon, however the nature of the neurotransmitters that underlie this phenomenon remain elusive.

Aims: We investigated whether blockade of major neurotransmitters at both the neuro-neuronal and neuro-muscular junction would block peristalsis induced by natural fecal pellet distension.

Methods: Video imaging of colonic wall movements was used to make spatio-temporal maps and determine the velocity of peristalsis. Propagation of artificial fecal pellets in the guinea-pig distal colon was studied in the presence of hexamethonium, atropine, ω-conotoxin (GVIA), ibodutant (MEN15596) and tetrodotoxin (TTX).

Results: Hexamethonium and ibodutant alone did not retard peristalsis (velocities in controls 1.98+/-0.05mm.sec^{-1}, n=32, hexamethonium 2.59+/-0.1mm.sec^{-1}, n=13, ibodutant 2.36+/-0.1mm.sec^{-1}, n=8, NS). ω-Conotoxin reduced velocity of propagation (0.67+/-0.2mm.sec^{-1}, P<0.01), but did not abolish peristalsis. Most interestingly, peristalsis was still preserved in a proportion of animals, in the combined presence of hexamethonium, atropine, ibodutant and ω-conotoxin receptor blockade, albeit in a staggered fashion with significantly reduced velocity (0.04+/-0.01 mm.sec^{-1}, n=3).

Discussion: The major finding of the current study is that colonic peristalsis can still occur after blockade of the major excitatory neuro-neuronal and neuro-muscular transmitters. Furthermore, the colon retained an intrinsic polarity in the presence of these antagonists and was only able to expel fecal pellets in an oral-to-anal direction. The mechanism underlying this polarized process remains incompletely understood, but in our hands does not require the activation of major excitatory neuro-neuronal and neuro-muscular transmitters.

POS-THU-022

NEURONAL LOSS IN THE MYENTERIC PLEXUS ASSOCIATED WITH THE ANTI-CANCER CHEMOTHERAPEUTIC AGENT IRINOTECAN

Stojanovska, V[1] and Nurgali K[1]

[1]College of Health and Biomedicine, Victoria University, Melbourne, Australia

Objective: Irinotecan is an effective chemotherapeutic agent used for the treatment of colorectal cancer. Nausea, vomiting and diarrhoea are the most common gastrointestinal side-effects experienced by patients undergoing treatment, persisting for months to years, and can be life-threatening. We hypothesised that these side-effects might be due to the damage to the enteric nervous system.

Method: Balb/c mice received intraperitoneal injections of irinotecan (30mg/kg/dose) three times a week for up to 14 days. The colon from sham-treated and irinotecan-treated mice was harvested at days 3, 7 and 14. Segments of the colon were dissected to expose the myenteric plexus. Wholemount preparations were double-labeled with a pan-neuronal marker (anti-PGP9.5 antibody) and with nitric oxide synthase (anti-nNOS antibody). The total number and nNOS-immunoreactive neurons were counted from four randomized images ($2mm^2$)/wholemount preparation. Colon cross sections (25µm) were labeled with anti-β-tubulin III to assess nerve fibre morphology.

Results: A significant decrease in the total number of neurons was observed at days 7 and 14 ($p<0.05$) in the irinotecan-treated group when compared to the sham-treated mice (n=3/group). No significant difference in the proportion of nNOS-immunoreactive neurons was observed at any time point between sham-treated and irinotecan-treated. Irinotecan induced changes in nerve fibre organisation and mucosal morphology as well as inflammation throughout the muscle layers of the colon.

Conclusion: Results showed that irinotecan induced a significant reduction in neurons at days 7 and 14, and induced inflammation throughout the colon. These effects could be associated with the side-effects experienced by patients undergoing chemotherapeutic treatment with irinotecan.

POS-THU-023

INTER-INDIVIDUAL DIFFERENCES IN BLOOD PRESSURE RESPONSES TO STRESSORS: A ROLE FOR THE BAROREFLEX?

Taylor CE[1,2], El Sayed K[2], Joyner MJ[3], Macefield VG[2,4]

[1]School of Science & Health, University of Western Sydney, Sydney, Australia; [2]School of Medicine, University of Western Sydney, Sydney, Australia; [3]Department of Anesthesiology, Mayo Clinic, Rochester, MN, US; [4]Neuroscience Research Australia, Sydney, Australia

Purpose: Evidence suggests that blood pressure (BP) reactivity to stressors predicts future hypertension. It has been hypothesized that inter-individual differences in BP reactivity may be explained by cardiovagal baroreflex sensitivity (cBRS), although results from previous studies are conflicting. Furthermore, the influence of sympathetic baroreflex sensitivity (sBRS) has not been tested. The aim is to examine the relationships between cBRS, sBRS and BP reactivity to mental and physical stressors. Methods: Muscle sympathetic nerve activity (MSNA), BP and heart rate responses to mental (Stroop test and mental arithmetic) and physical stressors (cold pressor, static handgrip exercise, and post-exercise ischaemia) were recorded in 22 healthy young individuals (18-26 yrs). Individuals who exhibited large increases in systolic BP (≥10 mmHg) were defined as 'responders' while those who exhibited increases <5 mmHg were defined as 'non-responders'. The relationship between systolic BP and R-R interval was used to determine cBRS via the sequence method. The threshold technique was used to assess sBRS, in which burst incidence was plotted against diastolic BP. Results: Mean ±SD cBRS was not significantly different between responders (14.2 ± 8) and non-responders (14.4 ± 4 ms/mmHg) to mental arithmetic (P>0.05). However, responders had significantly impaired sBRS (-1.2 ± 0.6) compared with non-responders (-1.9 bursts/mmHg; P=0.031). Correlation analyses revealed no significant relationships between cBRS or sBRS and reactivity to physical stressors (P>0.05). Conclusions: In young people low sBRS may be associated with greater BP reactivity to mental stress; responders have less capacity to buffer a stress-induced rise in BP via modulation of MSNA. (250 words)

POS-THU-024

VAGUS NERVE ACTIVITY, A GOOD NIGHT'S SLEEP, AND RECOVERY FROM ACUTE INFECTION.

Vollmer-Conna U [1], Cvejic E [1], Gunawardane U [1], Patel V[1], Lloyd AR [2]

[1] School of Psychiatry, University of New South Wales, Australia

[2] Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Australia

Under normal circumstances, "falling asleep" is preceded by changes in autonomic signaling, notably increased parasympathetic (vagal) activity and reduction of sympathetic drive with a concomitant reduction in heart rate (HR) and increase in HR variability (HRV). In a series of studies, we examined a role for nocturnal vagal activity in the experience of restorative sleep, and in mediating recovery from acute infection. HR was continuously monitored (via ECG) on two occasions in healthy students; in patients with chronic fatigue syndrome (CFS) and matched healthy controls; and in an initial sample of patients with acute infections. Questionnaires assessed demographic information, physical and psychological state, and sleep quality. Vagal activity was inferred from HRV parameters; actimetry served to monitor sleep/wake behaviour. The inflammatory marker, C-reactive protein, was quantified by ELISA in participants with acute infection. Repeated recordings of nocturnal HRV in healthy individuals showed a high level of reproducibility and were strongly linked to sleep quality. Compared to healthy participants, nocturnal HRV was significantly lower in patients with CFS and proved to be the best predictor of key sleep outcome variables. In patients with acute infections increased HRV was significantly associated with lower levels of C-reactive protein and shorter illness duration. This research confirmed that nocturnal vagal activation facilitates the recuperative effects of sleep and suggests a possible role for vagal activity in recovery from acute infection. (225 words)

POS-THU-025

DEVELOPMENT OF AXONAL PROJECTIONS TO THE URINARY BLADDER IN EMBRYONIC MOUSE

Wallace A S, Richardson E, Keast J R

Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Victoria, Australia.

The innervation of the urinary bladder is required for the micturition reflex and bladder sensory nerves are also involved in some pelvic pain conditions. Furthermore, these nerves are vulnerable to damage during pelvic surgery. Very little is known about the factors that determine growth of bladder nerves during the initial innervation of bladder tissues or following neurotrauma. Our aim was to define the spatiotemporal features of bladder innervation during embryonic development. Using C57Bl/6 male and female mice, we used immunofluorescence to document the patterning of various sensory and motor axonal markers from embryonic day (E) 12-18. This revealed a distinctive dorso-ventral pattern of innervation beginning at E13. Axons initially migrate through the outer detrusor muscle layer in a circumferential manner. Inwards radial migration to the lamina propria and urothelium occurs before the entirety of the detrusor is innervated and also occurs dorso-ventrally. At E16, blood vessels become innervated by tyrosine hydroxylase positive axons. By E18, each layer of the bladder is innervated extensively and appears nearly mature, however, the most dorsal regions of bladder continue to be more extensively innervated. Using phenotypic markers of axonal subtypes indicates distinct differences in the timing of entry into the primordial bladder tissue, the layers innervated and degree of innervation. In conclusion, as well as providing the first comparison of how bladder sensory and motor pathways are established, this data forms the basis of future studies on mechanisms driving axon growth and connectivity.

This study is part of the GUDMAP database initiative (www.gudmap.org).
(249 words)

POS-THU-026

CHARACTERISATION OF RELAXIN-3- AND RXFP3 RECEPTOR-POSITIVE NEURON POPULATIONS IN ADULT MOUSE BRAIN

Zhang C[1,2], Walker AW[1,2], Chua BE[1], Hossain MA[1,2], Rosengren KJ[3], Smith CM[1,2], Gundlach AL[1,2]

[1]The Florey Institute of Neuroscience and Mental Health and [2]Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria, Australia; [3]School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland, Australia

Relaxin-3 is a newly discovered peptide neurotransmitter implicated in the control of behavioural arousal, responses to stress and feeding. The present studies firstly examined the precise neuroanatomical distribution and relative number of neurons displaying relaxin-3-like immunoreactivity in male C57BL/6J mice (n=3). In line with previous preliminary studies, ~435 relaxin-3-positive neurons were present within the pontine nucleus incertus, ~70 within the pontine raphe, ~115 within the periaqueductal grey, and ~200 within the deep mesencephalic nucleus. Interestingly, the latter population consisted of two anatomically distinct sub-populations within either an area dorsal to the substantia nigra, or adjacent to the peripeduncular/subbraichial nuclei. Studies are ongoing to neurochemically identify/phenotype relaxin-3-positive neurons within these brain regions. Secondly, these studies sought to compliment ongoing pharmacological studies by determining the distribution of fluorophore-conjugated relaxin-3 analogues following their local infusion into the paraventricular nucleus of the hypothalamus (PVN), which is a region rich with relaxin-3's receptor, RXFP3 (relaxin family peptide 3 receptor). Mice were anaesthetised and unilaterally intra-PVN infused with 1µl of either the RXFP3 agonist 'R3/I5' (0.1 nmol) or antagonist 'R3(B1-22)R' (0.4 nmol), conjugated to fluorescein or Atto565, respectively. Mice were culled after 15 min, and peptides were observed to spread outside the borders of the PVN and into the anterior hypothalamic area, indicating a smaller injection volume is required for future PVN-specific targeting. These tissues will be further examined to determine whether peptide bound to RXFP3-positive neurons can be visualised, which will allow neurochemical phenotyping, as a specific anti-RXFP3 antibody is yet to be developed.

(249 words)

POS-THU-027

GENDER-SPECIFIC ROLE OF NEUROPEPTIDE FF RECEPTOR-2 SIGNALLING IN THE REGULATION OF ENERGY HOMEOSTASIS

Lei Zhang, I-Chieh J Lee, Herbert Herzog
Neuroscience Research Program, Garvan Institute of Medical Research, Sydney NSW Australia

The neuropeptide FF receptor 2 (NPFFR2) is highly expressed in the hypothalamus where it is activated by a set of RFamide peptides, however, its physiological function is unclear. Here we show that lack of NPFFR2 in female mice results in reduced adiposity, increased energy expenditure, body temperature and physical activity. The metabolic phenoptype of female NPFFR2-/- are associated with a significantly lower neuropeptide Y (NPY) mRNA expression in the arcuate nucleus (Arc), and the higher mRNA expression of pro-opiomelanocortin (POMC) in the Arc and tyrosine hydroxylase (TH) in the zona incerta. In contrast, male NPFFR2-/- mice show reductions in energy intake and adiposity but no other significant changes in metabolic parameters or neurotransmitter expression. Interestingly, when fed on a high-fat diet (HFD), both male and female NPFFR2-/- mice show greater weight and fat gain compared to gender-matched WT mice. Importantly, both male and female NPFFR2-/- on HFD also show significantly lower energy expenditure compared to WT mice, suggesting an impaired diet-induced thermogenesis. In support of that, HFD significantly increases UCP-1 and PGC-1αlevels in the brown adipose tissue of WT but not in NPFFR2-/- mice. The mechanism behind NPFFR2's control of energy expenditure and adaptive thermogenesis is likely to involve hypothalamic NPY pathways. This is particularly obvious in female mice where the HFD-induced decrease in Arc NPY expression observed in WT is absent in the NPFFR2-/- mice. In contrast, male NPFFR2-/- on HFD show even greater reduction in Arc NPY expression compared to WT mice, suggesting NPFFR2 may regulate diet-induced thermogenesis in male mice via NPY-independent pathway. Furthermore, HFD significantly increases the expression of thyrotropin-releasing hormone, a key energy regulating molecule expressed in secondary hypothalamic neurons downstream of NPY in male WT mice but failed to do so in male NPFFR2-/- mice. Taken together, these data demonstrate that NPFFR2 signalling plays important roles in the regulation of energy homeostasis and diet-adaptive thermogenesis, and NPFFR2 signaling exert these regulations via a gender-dependent central mechanisms.

POS-THU-028

NEURONAL IMAGING OF ENTERIC GANGLIA REVEALS SYNCHRONIZED OSCILLATORY FIRING IN A NETWORK OF MYENTERIC NEURONS DURING MURINE COLONIC MIGRATING MOTOR COMPLEXES

J.P. Zobel, L.Travis & N.J. Spencer
Discipline of Human Physiology, School of Medicine, Flinders University of South Australia.

Colonic migrating motor complexes (CMMCs) are cyclical contractions of the large intestine that occur rhythmically and propagate along the whole colon, leading to the propulsion of luminal contents. Recently, we identified the pattern generator that underlies CMMC rhythmicity was located within the myenteric plexus and/or muscularis externa, but how this pacemaker mechanism generates CMMCs is unknown. We developed a novel calcium imaging technique in which it is now possible to record the activation of multiple myenteric neurons during spontaneous and evoked CMMCs. The entire colon was pinned to the base of an organ bath, with mucosa and submucosal plexus removed. An electron multiplied-CCD camera and the calcium indicator Fluo-4 were used to record dynamic changes in cytosolic calcium within myenteric cell bodies of different ganglia at 35^{o}C. During the intervals between CMMCs, the two morphologically distinct populations of neurons (small soma; Dogiel Type 1) and large soma (Dogiel type 2) fired asynchronously. However, during CMMCs, the firing of both types of neurons became temporally phase-locked at 3.5 ± 0.2Hz (N=5) for periods of 5-20s. There was no significant difference in the temporal activation between Dogiel type 2 and type 1 neurons in neighboring ganglia (mean difference between peaks of calcium transients 32.2 ± 5.2ms; P=0.4; N=5). Hexamethonium abolished synchronized firing of all myenteric neurons (N=5). We show that the generation of the CMMC motor pattern involves the temporal synchronization of a large population of Dogiel Type 2 and Dogiel Type 1 myenteric neurons, involving multiple neighboring ganglia at constant oscillation frequency.

POS-THU-029

VERIFICATION OF C9ORF72 EXPANSION FREQUENCY IN ANOTHER AUSTRALIAN COHORT

MacMillan M[1], Huang Y[1, 2], Bartley L[1], Halliday G[1, 2].

1. Neuroscience Research Australia, Sydney, Australia. 2. The University of New South Wales, Sydney, Australia.

Introduction: A hexanucleotide repeat expansion in the C9orf72 gene has been identified as a major cause of frontotemporal dementia (FTD) in Australia (Dobson-Stone *et al.* Neurology 2012). This project aims to verify the C9orf72 mutation frequency, and associated clinical features, in another tertiary referral cohort.

Methods: 186 patients were recruited through the Frontier research clinic at Neuroscience Research Australia, 115 FTD (including 27 with behavioural variant FTD (bvFTD), 51 with primary progressive aphasia, 8 mixed or unspecified type FTD, and 29 atypical parkinsonian disorders), 64 Alzheimer's disease (AD), and 7 motor neuron disease (MND). A repeat primed PCR and fragment assay were used to detect the presence of the C9orf72 repeat expansion. Patients were identified as 'expansion-positive' if the hexanucleotide repeats numbered greater than 30 (Renton *et al.* Neuron 2011).

Results: The C9orf72 repeat expansion was detected in 4.3% of the cohort. The incidence of C9orf72 mutation was the highest in MND (42.9%), with the presence of a familial history or dementia during the disease course being significant risk factors, followed by 11.1% of those with bvFTD (33.3% of familial cases), 3.1% of those with AD, and none in other subtypes of FTD. In C9orf72 mutation carrier cases, bvFTD and MND are equivalent phenotypes (37.5%) with mutations less commonly seen in AD (25%).

Conclusions: The frequency of C9orf72 mutation in bvFTD is the same as in previous studies. However, the incidence of C9orf72 mutation is much higher in those with MND, which requires a larger cohort for validation. (248 words).

POS-THU-030

LOW-INTENSITY REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION FACILITATES PLASTIC REORGANISATION OF ABNORMAL CORTICAL PROJECTIONS AND UPREGULATES BDNF EXPRESSION

Makowiecki K[1,2], Harvey AR[1,3], Rodger J[1,2].
[1]Experimental and Regenerative Neuroscience, [2]School of Animal Biology, [3]School of Anatomy, Physiology and Human Biology, The University of Western Australia.

Purpose: Repetitive transcranial magnetic stimulation (rTMS) facilitates plasticity, and shows clinical promise in treating brain injury and psychiatric disorders. However, anatomical substrates and molecular mechanisms underlying rTMS effects remain poorly understood. We previously showed rTMS removes abnormal projections in a subcortical pathway of ephrin-A2A5$^{-/-}$ mice, a model of abnormal circuitry. However, clinically, rTMS is largely restricted to cortical regions, therefore it is important to establish whether rTMS also affects abnormal cortical projections. Methods: We compared rTMS effects on normal (wildtype;WT;n=14) and abnormal (ephrin-A2A5$^{-/-}$;n=16) corticotectal circuits. Mice received rTMS or sham (control) daily for 14 days. To visualize projections, fluorojade and fluororuby were injected into visual cortex. Mice were euthanized, brains cryosectioned and injection site and axon terminal-zone (TZ) location measured. TZs were quantified as normal or abnormal by comparing actual location to that predicted by injection sites, and ranked within each mouse. We also measured brain derived neurotrophic factor (BDNF) in visual cortex and superior colliculus (SC) after single and multiple stimulations. Results: rTMS significantly improved topography in the most disordered TZs in ephrin-A2A5$^{-/-}$ mice, without altering topographically normal TZs in ephrin-A2A5$^{-/-}$ or WT mice. BDNF levels significantly increased after a single stimulation for all groups, but only ephrin-A2A5$^{-/-}$ mice maintained increases in the SC at 14 days. Conclusion: Results suggest rTMS upregulates BDNF, maintaining a plastic environment conducive to beneficial reorganisation of abnormal connections. We provide the first direct evidence of cortical reorganisation and increase understanding of mechanisms underlying rTMS effects, an essential step towards optimizing its clinical use.

(word count: 250).

POS-THU-031

INVOLVEMENT OF EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK1/2)-P53-P21 AXIS IN MEDIATING NEURAL STEM/PROGENITOR CELL CYCLE ARREST IN CO-MORBID HIV-DRUG ABUSE EXPOSURE

Malik S, Saha R, Seth P
National Brain Research Centre, Manesar-122051, Gurgaon, India.

Adult neural stem/progenitors cells (NPCs) have been shown to be susceptible to viral infections such as HIV-1, which affects proliferation and differentiation of these cells. Illicit drug abuse continues to be a major predisposing co-factor in compounding HIV-1 neuropathology by leading to enhanced neurobehavioral abnormalities. Chronic exposure of HIV-1 protein, Tat and opioid, morphine decrease NPC proliferation, but the molecular mechanisms involved are still not defined. Using an *in vitro* model of human fetal brain-derived NPCs, we analyzed various cellular and molecular parameters involved in neural progenitor cell cycle progression. Using DNA content labeling and BrdU immunoassay, we observed enhanced propensity of NPCs to stall at the G1/S checkpoint in cell cycle after chronic Tat and morphine exposure. Immunoblotting studies revealed enhanced expression of p53 and cyclin dependent kinase (CDK) inhibitor, p21 in NPCs in response to Tat and morphine. p21 was found to be indispensable for Tat and morphine induced decrease in NPC proliferation as its knockdown abrogated the expected NPC cell cycle arrest. Expression of p21 was found to be governed via extracellular signal-regulated kinase-1/2 (ERK1/2) and p53 as their inhibition led to attenuation of Tat and morphine mediated decrease in p21 levels. This growth arrest in NPCs may lead to impaired ability of these cells to maintain their own pool as well as affect their differentiation potential. Our study provides key evidences for impairment in NPC biology, and emphasizes the need to fully understand individual and synergistic effects of HIV-1 and drugs of abuse on human CNS. (250 words)

POS-THU-032

CEREBRAL ENDOTHELIAL TIGHT JUNCTION STATUS HAS AN INFLUENCE ON THE ROLE AQUAPORIN-4 (AQP4) WATER CHANNELS PLAY IN CEREBRAL OEDEMA FOLLOWING BRAIN INJURY.

Modulating AQP4: effect on cerebral oedema following TBI.
MANDER, K.A., BURTON, J.L., TURNER, R.J., THORNTON, E., LEPPINUS, H.L., ZIEBELL, J.M., YOOL, A., & VINK, R*.

Purpose: Cerebral oedema is a life-threatening complication associated with traumatic brain injury (TBI) and thus interventions to adequately limit or reduce oedema are imperative. Recently, bidirectional water channels known as aquaporins (AQP) have been implicated in the physiological and pathological movement of water throughout the brain. Of most interest is the subtype aquaporin-4 (AQP4) due to its distribution at brain-water interfaces. Accordingly, we aimed to evaluate the effect of AQP4 modulation on oedema following TBI. Method: Adult male Sprague-Dawley rats were subjected to either focal cold lesion injury or moderate diffuse impact- acceleration injury. At 30mins post-injury, animals randomly received AQP4 agonist (AqpF026; 0.2 mg/kg IV), AQP4 antagonist (AqpB013; 0.8 mg/kg IV) or equal volume vehicle. Results: In focal injury, administration of the AQP4 agonist reduced oedema, whereas AQP4 antagonist treatment exacerbated oedema. However following diffuse TBI, AQP4 antagonist treatment ameliorated oedema whereas AQP4 agonist treatment had little effect. Conclusion: The type of AQP4 modulation required is dependent on injury type. This may be related to the status of blood-brain barrier tight junction proteins, which were disrupted in focal TBI but conserved in diffuse TBI. These findings support a role for AQP4 in oedema formation following TBI, indicating their potential as a therapeutic target.

POS-THU-033

INHIBITION OF MOTOR NEURON DEATH *IN-VITRO* AND *IN-VIVO* BY AN INTRACELLULAR DOMAIN FRAGMENT OF P75 NEUROTROPHIN RECEPTOR.

Matusica D[1], Alfonsi F[1], Turner B[2], Butler T[1], Skeldal S[1], Underwood CK[1], Mangelsdorf M[1], Matusica D[1], Alfonsi F[1], Turner B[2], Butler T[1], Skeldal S[1], Underwood CK[1], Mangelsdorf M[1], Coulson EJ[1].

1. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia
2. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia

The neurotrophin receptor ($p75^{NTR}$) is expressed by developing, injured and degenerating motor neurons, and can be activated by nerve growth factor (NGF) to mediate motor neuron apoptosis. $p75^{NTR}$ undergoes regulated intramembrane proteolysis (RIP), which regulates the activation of many if its signalling pathways. Here we report that inhibition of $p75^{NTR}$ extracellular proteolysis prevents NGF-induced death of cultured motor neurons. By contrast, inhibition of intracellular proteolysis of $p75^{NTR}$, preventing the generation of $p75^{ICD}$, promoted motor neuron death. We have recently reported that $p75^{ICD}$ is required for facilitating survival signalling by TrkA receptors and that a 29 amino acid juxtamembrane intracellular region of $p75^{ICD}$ mediated this effect. Here, we found that a peptide orthologue of this domain, c29, similarly facilitates of TrkB receptor signaling. c29 treatment inhibited NGF-induced death of cultured motor neurons, and enhanced the survival of motor neurons undergoing growth factor withdrawal in a BDNF, but not CNTF- and GDNF-dependent manner. Furthermore, in an injury paradigm, c29 treatment prevented the death of axotomised ulnar motor neurons *in-vivo*. More importantly treatment of $Sod1^{G93A}$ motor neuron disease mice with c29 for 60 days significantly enhanced the survival of spinal motor neurons in late disease. We conclude that proteolysis of $p75^{NTR}$ plays an important role in mediating NGF-mediated cell death and facilitating BDNF-dependent survival of motor neurons. These studies also suggest that modulation of BDNF/TrkB signaling by the c29 peptide mimetic of the $p75^{NTR}$ intracellular domain represents a novel strategy for treating motor neuron degeneration. (245)

POS-THU-034

CHARACTERISATION OF A NOVEL MODEL OF CHRONIC TRAUMATIC ENCEPHALOPATHY

McAteer KM, Corrigan F, Thornton E, van den Heuvel C, Vink R

Discipline of Anatomy and Pathology, School of Medical Sciences, University of Adelaide

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with contact sports and is associated with exposure to repetitive mild traumatic brain injury (mTBI). It is characterised by accumulation of phosphorylated tau within the frontal and temporal lobes and long term cognitive and behavioural deficits. Currently a number of in vivo models of repetitive mTBI are in development, however, there are discrepancies within the literature concerning their validity. This study aimed to characterise a model of repetitive mild TBI that accurately portrays both the short and long term clinical and histopathological features of CTE.

41 male Sprague-Dawley rats were subjected to either 0, 1 or 3 mTBIs induced by dropping a 450g weight from 1m onto a surgically adhered steel disc attached to the skull, spaced 5 days apart. Motor, cognition and anxiety like behaviour were assessed in the 6 weeks following injury and histopathological analyses undertaken at 24hrs.

3 mTBI rats showed both increased anxiety, with decreased exploratory behaviour on the Open Field ($p<0.05$). Mild learning deficits, with increased escape latency was seen on the Barnes Maze ($p<0.001$) with no associated motor deficits on the rotarod. Histologically, at 24 hrs post-injury, the 3 hit animals showed an increase in phosphorylated tau within neurons ($p=<0.01$) and increased microglial activation ($p<0.001$) within the cortex, directly under the impact site.

This is consistent with the clinical and pathological features of human CTE, and suggests this may represent a clinically relevant model that can be used to explore the underlying pathophysiological mechanisms. (249 words)

POS-THU-035

SYSTEMATIC REVIEW AND META-ANALYSIS OF ANTIDEPRESSANTS FOR THE TREATMENT OF ACUTE STROKE IN ANIMALS

McCann SK[1], Irvine C[2], Sena ES[2], Currie G[2], Egan K[2], Howells DW[1], Macleod MR[2]

1. The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia 2. Department of Clinical Neurosciences, University of Edinburgh, UK

There is mounting evidence to suggest that antidepressants may improve outcome in non-depressed stroke patients, independent of an effect on mood, and in animal models of stroke. The aim of this study was to assess whether there is evidence to suggest that the use of antidepressants in animal models of stroke has potential to ameliorate stroke outcome. Systematic review identified 44 studies, using 2115 animals, describing the use of antidepressants in a model of focal cerebral ischaemia. Random effects meta-analysis was used to estimate the overall effect of antidepressant treatment on infarct volume (primary outcome) and neurobehaviour. Meta-regression was used to investigate the impact of aspects of study design on treatment effects. Antidepressants were associated with an overall reduction in infarct volume of 27.3% (95%CI 20.7-33.8; n=58 observations) and improvement in neurobehaviour of 54.4% (46.6-62.2; n=98). Lower quality studies reported a significantly greater reduction in infarct volume. The antidepressant subtype, individual drug and type of neurobehavioural test used contributed significantly to between-study heterogeneity. Selective serotonin reuptake inhibitors (SSRIs) were the most commonly studied type of antidepressant. In the SSRI dataset, time of assessment, route of drug delivery and dosing procedure all accounted for a significant proportion of between-study heterogeneity. These data suggest antidepressants improve histological and neurobehavioural outcome in animal models of focal cerebral ischaemia and are in line with current, albeit limited, clinical data. However, heterogeneity exists, highlighting areas for improvement in study design and avenues for future experimentation. (240 words)

POS-THU-036

ROLE OF METALLOTHIONEINS IN NEUROPROTECTION AGAINST PARKINSON'S DISEASE

McLeary, FA[1], Kinder, J[1], Chung, R[2], Pountney, DL[1].

[1]Griffith Health Institute, Griffith University, Gold Coast, Australia. [2] School of Advanced Medicine, Macquarie University, Sydney, Australia.

Introduction: Parkinson's disease (PD) has hallmark symptoms of tremor and difficulty initiating movement. It is considered a disease of aging, primarily affecting individuals 65 years and above. Intracellular aggregates of the protein, alpha-synuclein, are the major pathological hallmark of PD and are linked to neurotoxicity. Raised extracellular copper has also been reported in PD and may be linked to alpha-synuclein aggregation. In this project, the potential neuroprotective role of the metal-binding proteins, metallothioneins (MT), has been explored in relation to copper-induced alpha-synuclein aggregation. The specific aims of the study were: 1) to investigate if metallothionein transfection can inhibit copper-induced alpha-synuclein aggregation in the SHSY-5Y neuroblastoma cell line; 2) to determine if up-regulation of endogenous expression of metallothionein by use of the synthetic glucocorticoid, dexamethasone, can prevent copper-induced aggregation of alpha-synuclein in SHSY-5Y cells. Methods: SHSY-5Y neuroblastoma cells were treated with 100µM copper to cause alpha-synuclein aggregation. Cells were either transiently transfected with MT-2-GFP or MT-3-GFP isoform expression vectors or treated with dexamethasone at various concentrations to induce endogenous MT expression. Immunofluorescence confocal microscopy was used to quantify alpha-synuclein aggregates under the different treatment conditions. Results: Transfection with MT-2-GFP or MT-3-GFP caused reduced copper-dependent alpha-synuclein aggregation. Dexamethasone treatment resulted in a significant (p, 0.01), dose-dependent up-regulation of MT expression in the SH-SY5Y cell line and a significant reduction in alpha-synuclein intracellular aggregates (p, 0.01). Conclusion: Metallothioneins (MT) show neuroprotective capability against copper-induced alpha-synuclein aggregation. Efficient MT up-regulation by the glucocorticoid, dexamethasone, was found, with a concomitant reduction in alpha-synuclein aggregation. (249 words).

POS-THU-037

CHARACTERISATION OF A SCHIZOPHRENIA-LIKE BVEHAVIOURAL PHENOTYPE IN A RODENT MODEL OF MATERNAL IMMUNE ACTIVATION IN EARLY VERSES LATE GESTATION.

Meehan CL 1, Frost J 1, Harms LR 1,6, Hodgson DM 1,6, Schall U 3,6, Todd J 2,6, Zavitsanou K 4,5,6, Shannon-Weickert C 4,5,6, Michie PT 2,6

1.Laboratory of Neuroimmunology, School of Psychology, University of Newcastle, Newcastle, Australia. 2.Functional Neuroimaging Laboratory, School of Psychology, University of Newcastle, Newcastle, Australia. 3.School of Medicine and Public Health, University of Newcastle, Newcastle, Australia. 4.School of Psychiatry, University of New South Wales, Sydney, Australia. 5.Neuroscience Research Australia, Australia. 6.Schizophrenia Research Institute, Australia.

Maternal immune activation (MIA) during gestation has been identified as a risk factor for schizophrenia. Recent evidence from a mouse model suggested that MIA in late gestation promoted schizophrenia-related cognitive dysfunction and altered NMDA receptor expression, whereas activation during early gestation was associated with changes in behaviours related to dopamine neurotransmission. The current study aimed to determine whether MIA in rats results in a reliable model of schizophrenia, with a focus on whether MIA during late gestation alters NMDA-related behaviours more so than early gestational activation.

Wistar rats were administered with either 4.0mg/kg of Polyl:C or saline on either gestational day 10 or 19. Prepulse inhibition (PPI) of the acoustic startle response and locomotion in response to MK-801 and amphetamine were examined in the adult offspring.

Early gestation MIA males were found to have reduced PPI in comparison to controls ($p<.05$) and both late gestation groups, driven primarily by reductions at long prepulse to pulse intervals and low prepulse intensities. Early gestation MIA males also showed a significant increase in locomotion in comparison to controls ($p<.05$) following administration of 0.1mg/kg of amphetamine but not at lower or higher doses. A trend was observed for late gestation MIA males to display increased locomotion following 0.3mg/kg of MK-801, but this difference was not statistically significant.

MIA in early, but not late gestation was associated with reduced sensorimotor gating in males. Sensorimotor gating deficits and increased sensitivity to amphetamine are observed in schizophrenia patients possibly indicative of a hyper-dopaminergic state. (247 words)

POS-THU-038

ROLE OF REGULATORY T CELLS IN DISEASE SEVERITY AND PAIN HYPERSENSITIVITY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

Duffy SS, Lees JG, Moalem-Taylor G
School of Medical Sciences, University of New South Wales, NSW 2052 Australia

Multiple Sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) characterised by motor dysfunction, which in severe cases may result in paralysis, and sensory disturbances; a common symptom being neuropathic pain. The pathology of MS is attributed to self-reactive CD4+ T cells against myelin, a T cell subtype susceptible to suppression by regulatory T (Treg) cells. In this study, we tested both CD4+ and Treg cell infiltration into the CNS during experimental autoimmune encephalomyelitis (EAE) using flow cytometry, pain behaviours during the course of EAE, and the effects of Treg cells on disease severity and pain sensitivity using transgenic DEREG (DEpletion of REGulatory T cells) mice. Results showed that clinically severe EAE corresponded to significantly increased CD4+ T cell infiltration into the CNS ($P < 0.05$), and this is preceded by mechanical and thermal pain hypersensitivity ($P < 0.05$). Further, we found that depletion of regulatory T cells resulted in significantly increased disease severity ($P < 0.0001$) of an otherwise mild form of EAE, although there appeared to be no effect on pain hypersensitivity. Overall, these findings suggest that Treg cells play a major role in the suppression of EAE, and that pain hypersensitivity is independent of EAE severity, however, further research is needed to ascertain the mechanisms underlying these observations. Nevertheless, the natural role of Treg cells in suppressing EAE may form the basis for the development of novel therapeutics in the treatment of MS in humans.

POS-THU-039

THE BETA-AMYLOID PROTEIN-INDUCED PHOSPHORYLATION OF CRMP-2 AT Thr555 SITE INHIBITS NEURITE OUTGROWTH AND AXONAL TRANSPORT IN ALZHIEMER'S PATIENTS

Mokhtar S.H.[1], Aui P.M. [1], Magee K[1], Cram D[1], Petratos S[2].
[1]Department of Anatomy and Developmental Biology, Monash University,Clayton 3800
[2]Department of Medicine, Central Clinical School, Monash University, Prahran victoria 3004.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder and the most common form of dementia. Evidence suggests that oligomeric Abeta can induce neuritic dystrophy, a pathological hallmark of AD. We set out to investigate how the Abeta peptide regulates the collapsin response mediator protein (CRMP-2). Post-mortem temporal and frontal lobe cortical AD (n=8 patients), multiple sclerosis (MS) (n=5), Huntington disease (HD) (n=4), fronto-temporal dementia (FTD) (n=9) and non-neurological disease control brain lysates (n=7) were analysed by immunoprecipitation, western blotting and immunostaining to identify CRMP-2 phosphorylation and inhibition of CRMP-2 binding to kinesin and tubulin. Coronal brain sections from Tg2576 mice (n=12) were also immunostained for phospho-Thr555 CRMP-2 and data compared with wild type brains (n=4). SH-SY5Y cells were also transfected with CRMP-2 phosphorylation-mutant constructs (n=5), treated with Abeta1-40 and Abeta1-42 (0.5, 1.0 and 10uM for 24h), to define which Abeta-mediated kinase may initiate phospho-CRMP-2-dependent neurite abnormalities. Human brain lysates showed increased PThr555CRMP-2 levels in AD when compared with FTD and control samples. Decreased kinesin and tubulin levels were observed after CRMP-2 immunoprecipitation from AD brain samples. Cortical and hippocampal neurons from aged Tg2576 mice (12-18 months) demonstrated substantial PThr555CRMP2 immunostaining colocalised with hyperphosphorylated tau. Moreover, SH-SY5Y cells transfected with the T555A phospho-CRMP-2 mutant construct generated larger processes, compared to cells transfected with other mutant constructs (sites phosphorylated by other kinases) following the provision of Abeta in culture. These data suggest that the phosphorylation of the Thr555 site on CRMP-2 may mediate Abeta-dependent neurite abnormalities associated with AD pathology. words).

POS-THU-041

GLUTATHIONE-S-TRANSFERASE OVEREXPRESSION IN THE NEURO2A CELL LINE PROTECTS AGAINST HYDROGEN PEROXIDE INDUCED CELL DEATH

Laurenti D[1], Maczurek A[1], Stone G[2], Ooi L[3], Gyengesi E[1], Münch G[1]

1. Molecular Medicine Research Group and Dept. of Pharmacology, School of Medicine, University of Western Sydney, Campbelltown, NSW, Australia
2. School of Computing and Mathematics, University of Western Sydney, Penrith, NSW, Australia
3. School of Biological Sciences, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, Australia

Oxidative damage is an event that precedes the appearance of the hallmark pathologies in several neurodegenerative diseases including Alzheimer's disease.. We have generated six different populations of the murine Neuro2A cell line with an up to 47-fold higher resistance by continuous exposure to hydrogen peroxide (H_2O_2) for 6 months. We hypothesize that these H_2O_2 resistant Neuro2A express neuroprotective genes at a higher level than the parental Neuro2a. In order to identify the most likely genes responsible for the acquired H_2O_2 resistance, we have correlated the normalized expression of specific genes based on the continuum of resistance responses with the LC_{50} value, yielding a certain number of candidate genes with a correlation coefficient > 0.8. Among those, two glutathione-S-transferases (GSTs), Gsta3 and Mgst1 emerged as promising candidates. To prove that they protect against H_2O_2 mediated toxicity, they were stably transfected in Neuro2a; overexpressing single clones were then tested for their H_2O_2 resistance. The GST clones were significantly more resistant to H_2O_2 toxicity, with the resistance correlating with the level of expression of the target gene. A possible mechanism could involve GST mediated detoxification of neurotoxic lipid peroxidation products such as acrolein or hydroxynonenal. Therapeutic approaches aimed at increasing glutathione (GSH) levels (resulting in increased GST activity) may therefore be protective for neurodegenerative diseases characterized by oxidative stress. (216 words)

Abstract theme: Neurodgenerative diseases

POS-THU-042

IDENTIFICATION OF ANTI-INFLAMMATORY COMPOUNDS FROM CINNAMON

Gunawardena D, Gyengesi E, Münch G

Molecular Medicine Research Group and Dept. of Pharmacology, School of Medicine, University of Western Sydney, Campbelltown, NSW, Australia

Activated microglia are characteristic hallmarks of Alzheimer's disease (AD). These activated microglia produce superoxide and nitric oxide, which contribute to (nitr)oxidative damage and neurodegeneration. Microglial activation can be ameliorated by anti-inflammatory antioxidants which can both redox-signal active oxygen free radicals and thus down-regulate nitric oxide and pro-inflammatory cytokine production. In a previous study, we have shown that cinnamon (*C. zeylanicum*) was one of the most potent anti-inflammatory foods out of 115 foods tested. The aim of this investigation was to determine the anti-inflammatory activity of *C. zeylanicum* and *C. cassia* extracts, to use sequential extraction and GC-MS to identify the volatile compounds, and determine their individual anti-inflammatory activity. When both cinnamon species were tested in lipopolysacharide and Interferon-γ activated RAW 264.7 macrophages using nitric oxide and TNF-α as readouts, they showed potent anti-inflammatory activity, particularly in the organic fractions, with the ethanolic fractions of both *C.zeylanicum* and *C.cassia* exhibiting IC_{50} values for NO below 24µg/mL and TNF-α below 45µg/mL. The most abundant compounds in both *C.zeylanicum* and *C.cassia* were *trans*- cinnamaldehyde *and o*-methoxy cinnamaldehyde. When the compounds were tested individually for their anti-inflammatory activity, the most potent compounds were *trans*-cinnamaldehyde, *p*-cymene, *o*- methoxy cinnamaldehyde, citral, estragole and *β*-caryophyllene which exhibited IC_{50} values for NO below 70µM. These findings indicate that both species, *trans*-cinnamaldehyde and *o*-methoxy cinnamaldehyde were responsible for most of the inflammatory activity of cinnamon. However, the extracts appeared to be more potent than the individual compounds, indicating the presence of unknown anti-inflammatory compounds or a synergistic effect. (247 words)

Abstract theme: Clinical Disorders and injury of the nervous system
Poster preferred

POS-THU-043

TEMOZOLOMIDE AND BEVACIZUMAB, NOT CALCIUM OVERLOAD, INDUCE MITOCHONDRIAL DYSFUNCTION IN HUMAN HIGH-GRADE BRAIN TUMOR.

Nanegrungsunk D[1,2], Apaijai N[2], Yarana C[2], Limpastan K[3], Watcharasaksilp W[3], Vaniyapong T[3], Chattipakorn N[2], Chattipakorn SC[2,4]

1. Department of Anesthesiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. 2. Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. 3. Neurosurgery Division, Department of Surgery, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. 4. Department of Oral Biology and Diagnostic Science, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.

Temozolomide (TMZ) and Bevacizumab (BEV) are two main chemotherapies for high-grade primary brain tumors. Although both agents effectively inhibit proliferating cancer cells *in vitro*, in clinical studies their poor efficacy might be due to the incompetence in disrupting the mitochondria. In addition, calcium overload is one of the crucial players in mitochondrial dysfunction, but its effects on mitochondrial function isolated from brain tumors have not been investigated. To address these issues, our present study investigated the effects of TMZ, BEV and calcium overload (200 µM) on the function of mitochondria isolated from low-grade and high-grade human primary brain tumor. Function of mitochondria was determined by measuring mitochondrial ROS production, mitochondrial membrane potential changes ($\Delta\Psi m$) and mitochondrial swelling. We found that calcium overload only caused mitochondrial dysfunction in low-grade, but not high-grade tumor, as indicated by increased mitochondrial membrane depolarization and increased mitochondrial swelling. Our finding suggested that mitochondria isolated from high-grade tumors were resistant to calcium overload. In contrast, TMZ and BEV interestingly caused mitochondrial swelling only in high-grade, but not in low-grade. (Figure 1) We also found the different underlying mechanisms between TMZ and BEV on mitochondrial dysfunction: TMZ caused a dramatically increase of ROS production, while BEV caused significantly $\Delta\Psi m$ dissipation. Our findings also suggest that combination of both drugs could be more effective in high-grade brain tumor therapy and that low clinically therapeutic success of both drugs in high-grade brain tumor treatment could be due to other factors rather than their action failure on brain tumors' mitochondria. (250 words)

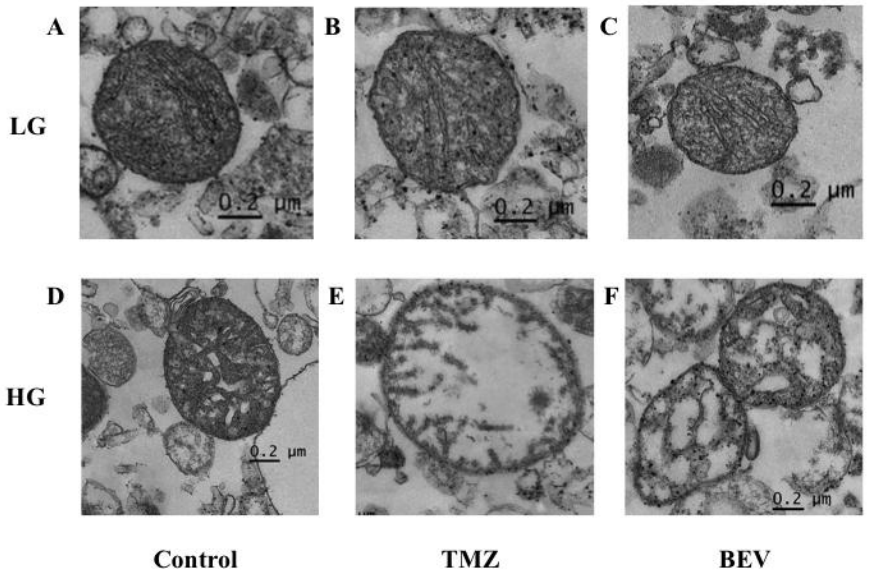

Figure 1: Effects of TMZ (300 µM) and BEV (2 mg/mL) on mitochondrial morphology of low-grade (LG) and high-grade (HG) brain tumor. TMZ: Temozolomide, BEV: Bevacizumab

POS-THU-044

INCREASED LIPID INFILTRATION INTO SKELETAL MUSCLE OF HSOD1G93A MICE.

Ngo, ST[1,2], Giuliani, KTK[1], Lee, K[1], Fogarty, MJ[1], Chen, C[1], Steyn, FJ[1], McCombe, PA[2,3]

1. School of Biomedical Sciences, The University of Queensland, Australia. 2. University of Queensland Centre for Clinical Research, The University of Queensland, Australia. 3. Department of Neurology, Royal Brisbane & Women's Hospital, Australia.

Increased body fat mass and BMI is associated with better survival in motor neuron disease (MND) patients. Moreover, high-fat feeding, resulting in increased adiposity, prolong lifespan in mouse models of MND. With evidence of defective lipid metabolism and preferential peripheral use of lipids during the course of disease, the provision of fat as an alternative energy substrate may explain the protective effects of high-fat diets in MND. To investigate substrate utilisation in MND, we assessed glycogen expression in skeletal muscle extracts, lipid infiltration into skeletal muscle fibre subtypes (I, IIA and IIB), and respiratory exchange ratio (RER; an indicator of substrate utilisation) in hSOD1G93A mice and wild-type age-matched controls. While we find no change in the expression of skeletal muscle glycogen in hSOD1G93A mice throughout the course of disease, we observe increased infiltration of free fatty acids into skeletal muscle fibres during the later stage of disease. Interestingly, assessment of RER relative to food intake and body composition reveals that hSOD1G93A mice appear to have impaired capacity to sustain energy supply from fat. Our results suggest that changes in energy substrate utilisation in hSOD1G93A mice occur during the later stages of disease. The impaired capacity of hSOD1G93A mice to sustain energy supply from fat may presumably be due to the depletion of fat stores in hSOD1G93A mice. Thus, supplementing changes in energy substrate utilisation may be beneficial in maintaining fat mass in MND. (233 words).

POS-THU-045

VITAMIN D2-ENRICHED BUTTON MUSHROOM (AGARICUS BISPORUS) EFFECT ON INFLAMMATION IN WILD TYPE AND APPSWE/PS1DE9 TRANSGENIC MICE

Bennett L[1], Kersaitis C[2], Macaulay L[3], Münch G[2,5,6], Niedermayer G[2], Nigro J[3], Payne M[4], Vallotton P[4] and Bird M[3]

1. CSIRO Preventative Health Flagship; Animal, Food and Health Sciences. 2. University of Western Sydney, School of Medicine. 3. CSIRO Preventative Health Flagship, Materials Science and Engineering. 4. CSIRO Mathematics and Information Sciences. 5. Molecular Medicine Research Group, University of Western Sydney. 6. Centre for Complementary Medicine Research, University of Western

Vitamin D deficiency is widespread, affecting over 30% of adult Australians, and increasing up to 80% for at-risk groups including the elderly. A reported positive relationship between Vitamin D status and cognitive performance suggests that restoring Vitamin D status might provide a cognitive benefit to those with Vitamin D deficiency. Two month old wild type (B6C3) and AD transgenic (APPSwe/PSEN1dE9) mice were fed a diet either deficient in Vitamin D2 or a diet which was supplemented with VDM for 7 months. Effects of the dietary intervention on memory were assessed pre and post-feeding. Brain sections were evaluated for amyloid β (Aβ) plaque loads, inflammatory biomarkers and microglia using immunohistochemical methods. VDM-fed wild type and AD transgenic mice displayed improved learning and memory, significantly reduced amyloid plaque load and elevated interleukin-10 in the brain. Resting/ramified microglial populations were also modified in the VDM-fed mice. These results suggest VDM might provide a dietary source of Vitamin D2 and other bioactives for preventing memory-impairment in dementia via the modulation of Aβ and inflammation.

POS-THU-046

INCREASED REACTIVE OXYGEN SPECIES AND OXIDATION OF WHITE MATTER PROTEINS IN CNS VULNERABLE TO SECONDARY DEGENERATION

O'Hare Doig RL[1,2,3], Bartlett CA[1,2], Hodgetts S[1,3], Fitzgerald M[1,2].

[1]Experimental and Regenerative Neurosciences, The University of Western Australia, Crawley, WA 6009, Australia, [2]School of Animal Biology, The University of Western Australia, Crawley, WA 6009, Australia, [3]School of Anatomy, Physiology and Human Biology, The University of Western Australia, Crawley, WA 6009, Australia.

Following partial injury to the CNS, cells beyond the initial injury site undergo secondary degeneration, with oxidative stress thought to contribute to further loss of neurons, compact myelin and function. However, increased reactive oxygen species and subsequent oxidative changes in proteins are yet to be shown in white matter exclusively vulnerable to secondary degeneration. We have demonstrated changes in a suite of oxidative stress indicators in spared ventral optic nerve vulnerable to secondary degeneration following partial dorsal nerve transection**.** In particular, we demonstrated significant increases in staining of fresh frozen sections with 2',7'-dichlorofluorescein (H_2DCF), a non-specific probe to detect a broad range of reactive oxygen and nitrogen species, at Day 1 following injury ($p \leq 0.05$). Dihydroethidine (DHE), used to detect superoxide, was significantly increased at day 7 after injury, in cell bodies throughout the nerve, including areas vulnerable to secondary degeneration ($p \leq 0.05$). Amplex UltraRed staining, used to assess H_2O_2, was significantly increased at 1 and 3 days following injury ($p \leq 0.05$). Redox proteomics analyses were conducted to determine the consequences of increased reactive oxygen species on proteins in optic nerve vulnerable to secondary degeneration. Initial pilot data indicates that there is an increase in the number of oxidised protein sequences in optic nerve at day 7 following injury (53 sequences), when compared to normal optic nerve (46 sequences). Identification of reactive oxygen species associated with oxidised proteins, may enable rational design of anti-oxidant therapies to reduce oxidative stress, and associated dysfunction and death of neurons and glia in CNS vulnerable to secondary degeneration. (250 words).

POS-THU-047

ACTIVATION OF AN ALTERNATIVE MITOPHAGIC PATHWAY MAY COMPENSATE FOR A LOSS OF PARKIN-MEDIATED MITOPHAGY

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

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

Mutations in the E3 ubiquitin ligase Parkin have been shown to cause autosomal recessive, early-onset Parkinson's disease (PD). Parkin plays an important role in the maintenance of mitochondrial function and morphology, thus mitochondrial dysfunction has been implicated as the main cause of Parkin-related PD. Recently, we identified a homozygous *Parkin* mutation in an asymptomatic mutation carrier who lacked functional Parkin but displayed no clinical manifestation of PD.

We examined mitochondrial function by measuring ATP production and cellular viability under mitochondrial stress in the carrier-derived fibroblasts. We treated the cells with the mitochondrial uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and assessed mitophagic clearance by three methods; measurement of citrate synthase activity, mitochondrial DNA content and the co-localisation of autophagosomes with mitochondria.

Surprisingly, we observed normal mitochondrial function in carrier-derived fibroblasts despite a lack of Parkin protein. There was no compensation for Parkin-mediated ubiquitination of mitofusin 2 and basal autophagy was unaltered. When the carrier-derived fibroblasts were exposed to CCCP they displayed normal activation and progression of CCCP-induced mitophagy.

The results indicate that a Parkin-independent alternative mitophagic pathway is activated in the homozygous *Parkin* mutant carrier's cells, which maintains a pool of healthy mitochondria. Given the function of Parkin, restoration of mitophagy by compensatory Parkin-independent pathway(s) is likely preventing the clinical manifestation of Parkin-related PD in the homozygous mutation carrier. The identification of this alternative mitophagy pathway provides a potential therapeutic avenue for the treatment of mitochondrial dysfunction in Parkin-related PD. (238 words)

POS-THU-048

MILD HYPOXIA CAN PREVENT INJURY-INDUCED INFLAMMATORY PROCESSES IN THE NEONATAL RAT BRAIN

Parmar J[1], Jones NM[1].

1. Department of Pharmacology, School of Medical Science, University of New South Wales, Sydney, NSW, Australia.

Inflammation plays an important role in the pathophysiology of neonatal hypoxic-ischemic (HI) brain injury. Studies have shown that hypoxic preconditioning (HP) can ameliorate brain damage by various mechanisms, but its effects on inflammation remain unknown. Postnatal day 6 (P6), Sprague-Dawley rat pups were divided into normoxia and hypoxia (8% oxygen, 3hrs) groups. On P7 some pups from both the groups underwent a permanent unilateral right carotid artery occlusion followed by hypoxia (8% oxygen, 3hrs) while under 1.5% isofluorane anesthesia. The remaining pups underwent sham surgery without occlusion. Animals were sacrificed 5 days later and fixed tissue was used to examine the changes in neurons, astrocytes and microglia in the cortex whereas fresh tissue was collected to determine levels of proinflammatory cytokines using ELISA. There was ~ 37% loss of cortical neurons following HI injury, which was improved by ~18% when HP was given prior to HI (p<0.05, ANOVA). There was a 100% increase in area of astrocyte staining after HI injury compared to control. HP before HI was able to prevent the gliosis and area of glial stainingback to the control level (p<0.05). HI caused an ~420% increase in the number of activated microglia compared to control and this was markedly reduced by HP to ~250%. Compared to control, interleukin-1β levels in the cortex increased by 45% after HI, but HP prior to HI was able to reduce this level to 30%. Therefore, the protective actions of HP appear to involve a reduction in inflammatory mediators. (Word count 246)
mediators. (Word count 246)

POS-THU-049

EFFECT OF ACTIVE IMMUNISATION WITH MYELIN BASIC PROTEIN AND ITS ALTERED PEPTIDE LIGAND ON CENTRAL NEUROPATHIC PAIN IN UN-INJURED AND SPINAL CORD-INJURED LEWIS RATS

Perera C J[1], Lees J G[1], Duffy S S[1], Apostolopoulus V[2], Moalem-Taylor G[1]

[1]School of Medical Sciences, University of New South Wales, Sydney, NSW, 2052, Australia

[2]VAConsulting Services, Melbourne, VIC

Neuropathic pain is a common symptom in patients with spinal cord injury and multiple sclerosis (MS), and adversely affects life quality. Myelin Basic Protein (MBP) induces autoimmunity and has been found in cerebrospinal fluid of MS patients. Myelin-derived altered peptide ligands (APL) have been shown to divert immune reaction towards a beneficial anti-inflammatory response. Considering the involvement of immune responses in neuropathic pain, we studied the effect of treatment with APL on mechanical and thermal pain hypersensitivity in the hindpaws of Lewis rats following immunisation with MBP or after spinal cord hemisection at level T13. Normal rats were injected subcutaneously with either weakly encephalitogenic MBP (MBP_{87-99} 200µg, n=6), APL+MBP (cyclo (87-99) [A^{91}, A^{96}]MBP_{87-99}, 250µg + MBP_{87-99} 200µg, n=6) in complete Freund's adjuvant (CFA) (1mg/mL) or CFA only (control,1mg/mL, n=6). Rats immunised with MBP developed significant mechanical and thermal pain hypersensitivity that was significantly reduced by combined injection of APL and MBP ($P<0.0001$, days 10-27 post-immunisation). Spinal cord-injured rats were immunised intradermally with either MBP (cyclo MBP_{87-99} 250µg, n=6), APL (cyclo(87-99)[A^{91},A^{96}]MBP_{87-99}, 250µg,n=6) in CFA (1mg/mL, n=6) or with CFA as control. Spinal cord-injured rats immunised with APL exhibited only a transient improvement in mechanical withdrawal threshold ($P<0.0001$, day 4) and thermal withdrawal latency ($P<0.01$, day 7) as compared to MBP/CFA treated rats. These results suggest that active immunisation with MBP-derived APL has a differential effect on neuropathic pain depending on the model used; however, the exact underlying mechanisms need to be further investigated. (243 words)

POS-THU-050

MAINTENANCE AND ADAPTATION OF THE NEUROMUSCULAR SYNAPSE UNDER AUTOANTIBODY CHALLENGE AND DRUG TREATMENT.

Ghazanfari N[1], Morsch M[1], Tse N[1], Reddel SW[2], Klaus V. Toyka[3] Phillips WD[1].

1. Physiology and Bosch Institute, University of Sydney, Sydney, New South Wales 2006, Australia. 2. Department of Molecular Medicine, Concord Hospital, Concord, New South Wales 2139, Australia. 3. Department of Neurology, University of Würzburg, Josef-Schneider-Str.11, 97080 Würzburg, Germany.

During development Muscle Specific Kinase (MuSK) functions to coordinate the clustering of postsynaptic acetylcholine receptors (AChR) and presynaptic nerve terminals at the mouse neuromuscular junction (NMJ). In a subset of myasthenia gravis patients muscle weakness is linked to MuSK autoantibodies. We have studied the effects of injecting IgG from anti-MuSK-positive patients (AM IgG) into C57Bl6J mice. Mice receiving daily injections of AM IgG developed severe weakness over 15 days. In fibres of the diaphragm muscle, the amplitudes of the nerve-evoked endplate potential (EPP) and the quantal amplitude were reduced. Reduced quantal size could be explained by progressive wastage of AChRs from the postsynaptic membrane scaffold. Immunostaining revealed reduced postsynaptic membrane staining for MuSK, activated Src, phosphorylated AChR and rapsyn. These results suggest that MuSK autoantibodies suppress the MuSK signalling pathway at the adult NMJ and an ongoing role of MuSK to control the turnover of AChRs in the postsynaptic membrane scaffold. The EPP recordings also suggest that AM IgG injections inhibited the adaptive increase in quantal content that would normally occur (over several days) following a reduction in postsynaptic AChR density. We tested the effects of drugs often used to treat myasthenic disorders in this mouse model of anti-MuSK myasthenia gravis. Pyridostigmine (a cholinesterase inhibitor and first line treatment) perversely exacerbated synapse disassembly, most likely by prolonging postsynaptic AChR responses. In contrast, 3,4-diaminopyridine increased quantal content, and hence EPP amplitude. Albuterol, a β2-adrenoceptor agonist, reduced weakness but without preventing the loss of postsynaptic AChRs in the muscles examined. (248 words).

POS-THU-051

EVALUATING THE ROLE OF APP96-110 AS A NOVEL THERAPEUTIC AGENT FOLLOWING TRAUMATIC BRAIN INJURY

Plummer SL[1], Thornton E[1], Corrigan F[1], Vink R[1], Cappai R[2], Van den Heuvel C[1]

1. Adelaide Centre for Neuroscience Research, The University of Adelaide, South Australia, Australia. 2. Department of Pathology, The University of Melbourne, Victoria, Australia.

Traumatic Brain Injury (TBI) is estimated to surpass many diseases as the leading cause of death and disability by 2020. Survivors are often left with considerable neurological damage, occurring through a cascade of deleterious physiological events over subsequent days. The Amyloid Precursor Protein (APP) has been identified as offering neuroprotective properties following TBI. Our research has shown that intracerebroventricular administration of soluble amyloid precursor protein alpha and its derivative, the APP96-110 peptide, improves functional outcome and protects against neuronal injury following TBI in rodents. The current study investigated the effects of intravenous (IV) administration of 2.5µM, 25µM and 250µM APP96-110 post TBI in rats, to identify an optimal dose. 47 male Sprague-Dawley rats received moderate-severe diffuse TBI, before administration of APP96-110 at 30 mins post - injury. Rats were assessed over 7D on the rotarod, with brain tissue then examined histologically for axonal injury (AI) within the corpus callosum. Following injury, a significant improvement in motor function was evident in rats receiving 25µM and 250µM APP96-110. Their motor function remained consistently higher than vehicle controls and 2.5µM rats over 7D, reaching sham levels by day 5. At 7D post trauma, the 2.5µM, 25µM and 250µM APP96-110 treated animals all showed reduced AI when compared to vehicle controls. This suggests that the 2.5µM dose was only sufficient to improve histological, but not functional outcome, in comparison to the 25µM and 250µM doses. This suggests that APP96-110 is a clinically relevant therapeutic option in a model of moderate-severe TBI. (246 words)

POS-THU-052

BDNF TRANSCRIPTS ARE UPREGULATED IN THE FRONTAL CORTEX BY TESTOSTERONE REMOVAL DURING ADOLESCENCE IN MALE MACAQUES AND RATS

Purves-Tyson TD[1,2,3], Allen KM[1,2,4], Fung SJ[1,2,4], Rothmond DA[124], Shannon Weickert C[1,2,4].

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

Adolescence, a time of increasing circulating testosterone in males, is the age with the highest rate of onset of schizophrenia. Changes in sex steroids are able to modulate cognition and decline in cognition is a debilitating symptom in schizophrenia. Brain derived neurotrophic factor (BDNF) is a neurotrophic factor required for cortical development and cognitive function and in schizophrenia BDNF is decreased in the frontal cortex. We investigated if BDNF transcripts are modulated by gonadectomy in the cortex of adolescent male monkeys and rats. Male rats (45 days old; n=15/group) and rhesus macaques (2.5 years; n=6/group), prior to adolescent testosterone surge were gonadectomised or left intact for 2 weeks or 2 years (i.e. young adulthood), respectively. Some rats were given replacement testosterone for 2 weeks. BDNF transcripts were measured in the prefrontal cortex by in situ hybridization (monkey) or qPCR (rats). In the monkey, BDNFII and BDNFVI transcripts were increased in frontal cortex following gonadectomy and BDNFVI expression was negatively correlated with circulating testosterone levels. In rat frontal cortex, gonadectomy increased BDNFIIa and there was a trend for this to be prevented by testosterone. BDNFVI and BDNFIII mRNAs were decreased by testosterone replacement relative to the gonadectomised group. The ability of testosterone to modulate BNDF expression may be conserved across species. We conclude that changes in circulating testosterone at adolescence may contribute to changes in cortical plasticity via decreasing cortical BDNF transcripts. This may impact adolescent cortical development in individuals susceptible to schizophrenia. (242 words)

POS-THU-053

NEUROINFLAMMATION IN MULTIPLE SYSTEM ATROPHY IS INFLUENCED BY PATHOLOGICAL ALPHA-SYNUCLEIN AGGREGATES

Radford R[1], Rcom-H'cheo-Gauthier A[1],Wong M[1], Eaton ED[2], Quilty M[2], Meedeniya A[1], Vickers JC[2], Gai WP[3], Guillemin G[4], Norazit A[5],West AK[2], Dickson TC[2],Chung R[4], Pountney DL[1]

[1]Griffith Health Institute, Griffith University, Gold Coast, Australia. [2]Menzies Research Institute Tasmania, University of Tasmania, Hobart, Australia.[3]Centre for Neuroscience, Flinders University of South Australia, Adelaide, Australia.[4]School of Advanced Medicine, Macquarie University, Sydney, Australia. [5] Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Introduction: Multiple system atrophy (MSA) closely resembles Parkinson's disease (PD) clinically, but with a range of autonomic signs. However, unlike PD that displays primarily neuronal pathology, MSA exhibits widespread astrogliosis and the occurrence of α-synuclein (α-syn) glial cytoplasmic inclusions (GCIs) in mature oligodendrocytes. To investigate the relationship between α-syn inclusions and neuroinflammation in MSA, we conducted quantitative morphometric analysis on MSA cases, and cell culture and animal model studies. Methods: Using Imaris software, we obtained "skinned" three-dimensional models of GFAP-positive astrocytes in MSA and normal tissue (n = 75) from confocal z-stacks and measured the astrocyte process length and thickness and radial distance to GCI. Results: Astrocyte activation results in highly ramified astrocyte morphology with extended and thickened processes. Astrocytes proximal to GCI-containing oligodendrocytes (r < 25 µm) had significantly (*p*, 0.05) longer and thicker processes than distal astrocytes (r > 25 µm), with a reciprocal linear correlation (m, 90 μm^2) between mean process length and radial distance to the nearest GCI (R^2, 0.7). In primary cell culture studies, α-syn addition caused ERK-dependent activation of rat astrocytes and perinuclear α-syn inclusions in mature (MOSP-positive) rat oligodendrocytes. Activated astrocytes were also observed in close proximity to α-syn deposits in a unilateral rotenone-lesion mouse model. Moreover, unilateral injection of MSA tissue-derived α-syn into the mouse medial forebrain bundle resulted in widespread neuroinflammation in the α-syn-injected, but not sham-injected hemisphere. Conclusion: Taken together, our data suggests that localized extracellular concentrations of α-syn may underlie both astrocyte and oligodendrocyte MSA pathological features. (248 words).

POS-THU-054

CENTRAL SEROTONERGIC NEURON DEFICIENCY IN A MOUSE MODEL OF ZELLWEGER SYNDROME

Rahim RS[1], Meedeniya ACB[2], Crane DI[1]

[1]Eskitis Institute for Drug Discovery, and School of Biomolecular and Physical Sciences, and [2]Apoptosis Research Group, School of Medical Science, Griffith Health Institute, Griffith University, Qld, Australia.

Zellweger syndrome (ZS) is a severe peroxisomal disorder caused by mutations in peroxisome biogenesis, or PEX, genes. A central hallmark of ZS is abnormal neuronal migration and neurodegeneration, which manifests as widespread neurological dysfunction. The molecular basis of ZS neuropathology is not well understood. Based on earlier findings of reduced expression in brain of *tph2*, which encodes the rate-limiting enzyme of serotonin (5-hydroxytryptamine, 5-HT) synthesis, we have investigated the serotonergic system using a mouse model of ZS neuropathology with conditional brain inactivation of the *PEX13* gene[1]. We show that PEX13 brain mutants display a range of changes that reflect an abnormal serotonergic development and function – decreased levels of TPH2, dysmorphic 5-HT positive neurons, abnormal migration of 5-HT neurons, and dystrophic serotonergic fibers. The raphe nuclei region of PEX13 brain mutants also display increased levels of apoptotic neurons and reactive, inflammatory gliosis. Given the role of the serotonergic system in brain development and motor control, dysfunction of this system may account in part for the observed neurological changes of PEX13 brain mutants. (171 words)

[1]Muller CC, Nguyen TH, Ahlemeyer B, Meshram M, Santrampurwala N, Cao S, Sharp P, Fietz PB, Baumgart-Vogt E, Crane DI (2011) PEX13 deficiency in mouse brain as a model of Zellweger syndrome: abnormal cerebellum formation, reactive gliosis and oxidative stress. Dis Model Mech, 4**,** 104-19.

POS-THU-055

DOES EXERCISE TRAINING AFTER SPINAL CORD INJURY INFLUENCE THE PHYSIOLOGICAL PROPERTIES OF SPINAL INTERNEURONS?

Michelle M Rank[1], Jamie R Flynn[1], Lynda R Dunn[1], Mary P Galea[2], Robin Callister[1], Robert J Callister[1]
[1]School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW
[2]Melbourne School of Health Sciences, University of Melbourne, VIC, Australia

Following incomplete spinal cord injury (SCI) axon sprouting and synaptogenesis occur in the vicinity of the lesion, and are enhanced by exercise. The effect of exercise on the properties of neurons and newly formed synapses is unknown. Here we examine the influence of different doses (3, 6 or 9 weeks) of exercise (treadmill training) on the intrinsic and synaptic properties of interneurons in the vicinity of an SCI. Adult male mice (C57BL/6; ~P63) received a spinal hemisection (T9-10) and were divided into untrained (no exercise) and trained (treadmill exercise, 2 x 10 min, 5 d/wk) groups. Control mice were not injured and received no exercise training. After completing their training, mice were overdosed (ketamine 100mg/kg, ip) and horizontal spinal cord slices (T5-T13) were prepared for whole-cell patch clamp recording. Intrinsic properties including resting membrane potential, input resistance, rheobase current, and action potential threshold changed over time after SCI, however, this was independent of training. Spontaneous excitatory postsynaptic currents (sEPSCs) and dorsal column evoked EPSCs were recorded to examine synaptic connections. sEPSC rise and decay times increased while sEPSC frequency decreased after 6 and 9 wks of exercise training. The amplitude and charge of evoked EPSCs increased after 3 wks of training and was maintained with longer training durations. These data indicate that intrinsic membrane properties are altered independent of exercise training after SCI, whereas longer periods of exercise selectively strengthen some synaptic properties in neurons within the vicinity of an SCI. (241 words)
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POS-THU-056

EFFECTS OF HIGH AND LOW RADIATION DOSE ON GENE EXPRESSION CHANGES IN CULTURED MURINE BRAIN ENDOTHELIAL CELLS

Raoufi-Rad N[1], Gupta KK [2], Stoodley M[1].

1. Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia, 2. Genesis Cancer Care, Radiation Oncology Department, Macquarie University Hospital, Sydney, NSW, Australia.

Brain arteriovenous malformations (AVMs) are abnormal connections between arteries and veins and are the leading cause of hemorrhagic stroke in children and young adults. Safe and effective treatment of large and deep AVMs remains challenging, therefore new treatment methods are required. It is proposed that stereotactic radiosurgery could be used to selectively alter AVM endothelial cell phenotype, allowing targeted molecular therapies that stimulate thrombosis. We have previously shown that irradiation of endothelial cell cultures with 25Gy results in up-regulation of leukocyte-endothelial cell adhesion molecule 2 (E-selectin) and leukocyte-endothelial cell adhesion molecule 3 (P-selectin). In this study we test the hypothesis that a lower dose of radiation is similarly effective at causing up-regulation in E-selectin and P-selectin expression.

Murine brain endothelial cell cultures were treated with 15Gy (n = 4) or 25Gy (n = 4) using a linear accelerator; non-irradiated cells were used as controls (n = 4). Quantitative real-time PCR was used to measure the relative gene expression at 24, 48, 72 and 96h after radiation. Genes encoding for E-selectin and P-selectin were found to be up-regulated post-radiation with either 15Gy or 25Gy. Maximum gene expression of E-selectin and P-selectin was observed at 24h. There was no significant difference between the two doses. These data support the hypothesis that a 15Gy dose is as effective as a 25Gy dose in inducing E-selectin and P-selectin expression. This is important from a clinical perspective because lower doses of radiation will be safer for treatment of large AVMs. (245 words).
(245 words).

POS-THU-057

REDUCED WHOLE BRAIN VOLUME AND REGIONAL SPECIFIC ARCHITECTURAL CHANGES IN A MOUSE MODEL OF DRAVET SYNDROME.

Richards, K1, Retchford, AR1, Reid, CA1 ** and Petrou S1,2,3

1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia. 2. Department of Anatomy and Neuroscience, The University of Melbourne, Victoria, Australia. 3. Centre for Neural Engineering, The University of Melbourne, Victoria, Australia.

Dravet Syndrome is a devastating disease with clinical symptoms including severe epilepsy, febrile seizures, movement disorder and reduced life span. Homozygous mutations in *SCN1B*, which encodes the beta subunit of the voltage-gated sodium channel, can cause Dravet syndrome. We have engineered a homozygous Scn1b mutant mouse model that shares phenotypic and pharmaco-sensitivity with Dravet Syndrome. In addition to its conducting role, Scn1b has cell adhesion properties. Consitent with this, cellular studies have identified a decrease in the size and arborisation of hippocampal subiculum neurons in the Scn1b mutant mouse that are propsed to underlie excitability. Whether dysfunction in this cell adhesion role has a more global influence on brain connectivity is not known. Aim: We hypothesized that C121W homozygote mice have altered brain connectivity. Methods/Results: Diffusion MRI data was acquired for *ex-vivo* brains aged P16 using a 16.4T system, processed using constrained spherical deconvolution tractography. Track-density imaging informed structural connectivity at 20µm isotropic resolution. Analysis of whole-brain diffusivity suggested a 16% reduction in brain volume for Scn1b mutant compared to wild type mice; however region analysis (i.e. hippocampus and cerebellum) indicated the change was not congruent. Neuron cell density was unchanged further indicating network disruption in C121W mice. Conclusion: The study's findings support our hypothesis and suggest the dysfunction in Scn1b impact is severe in affected regions but this is not a global change. (224 words)

POS-THU-058

HARMFUL EFFECTS OF “FAST FOODS” ON ENTERIC NEURONS

Rivera, LR[1], Leung C[2], Pustovit RV[1], Furness JB[1]

1. Department of Anatomy and Neuroscience, The University of Melbourne, Australia
2. Department of Gastroenterology, Austin Health, Australia

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. It has a growing prevalence and has been predicted to become the leading cause of cirrhosis and end-stage liver disease. Compounds known as advanced glycation end products (AGEs) have been shown to exacerbate liver injury. AGEs are complexes of reducing sugars and proteins formed when proteins are overheated in the presence of fats and sugars (e.g., by deep frying). AGEs are low in traditional diets, but are common when diets include so-called fast foods. Impaired gastrointestinal function has been implicated in the development of NAFLD, but there is very little known about the effects of NAFLD and AGEs on enteric neurons. Therefore, this study aimed to assess damage to myenteric neurons within the ileum, caecum, and colon of C57bl/6 mice with NAFLD induced by high fat high cholesterol (HFHC), and HFHC baked diets (increases AGEs content fivefold) for 20 weeks. Quantitative immunohistochemistry was used for the investigation of specific neuronal markers. There was a significant decrease in the number of Hu and nitric oxide synthase (NOS) immunoreactive neurons in the HFHC and HFHC baked groups compared to control. In addition, there was a significant increase in Hu translocation to the nucleus, granular Hu deposits in the cytoplasm and the presence of cytoplasmic vacuoles (indices of damage) in the HFHC and HFHC baked groups compared to control. This work indicates that HFHC and HFHC baked diets result in enteric neuropathy that may underlie impaired gastrointestinal function in NAFLD.

POS-THU-059

EXPLORING THE MECHANISMS FOR STRUCTURAL REORGANISATION OF CORTICAL CIRCUITS FOLLOWING RTMS: REGULATION OF THE EXPRESSION OF SEROTONIN RECEPTOR AND TRANSPORTER GENES

Penrose-Menz, MA, Rodger, J

Experimental and Regenerative Neuroscience, University of Western Australia, Crawley 6009, Western Australia.

Repetitive transcranial magnetic stimulation (rTMS) is a promising therapy to promote recovery in a range of neurological conditions. In addition, clinical trials combining rTMS with drugs targeting serotonergic signalling suggest synergistic effects in the treatment of depression. However, progress has been limited by a lack of understanding of the molecular and cellular mechanisms underpinning rTMS effects. We have used in vitro and in vivo models to elucidate changes in serotonergic gene expression following rTMS. First, we delivered a single session of repetitive magnetic stimulation (10 minutes;18 mT; 6-9Hz biomimetic frequency) to primary cultures of mouse cortical neurons. Total RNA was extracted 5 hours later and PCR array analysis revealed a significant ($p<0.05$) increase in the expression of VMAT1, a vesicular monoamine transporter with high affinity for serotonin. To further investigate changes in the serotonergic system, normal adult C57Bl/6j mice received a single rTMS stimulation session (as above) delivered to the dorsal cranium. Controls underwent the same procedure with the stimulator switched off (n=6 per group). Six hours later, mice were transcardially perfused and brains sectioned for immunohistochemistry. We found significant upregulation of the serotonin transporter 5HTT and downregulation of the serotonin receptor 1A in cortex of stimulated mice ($p<0.05$ vs controls). An increase in the expression of VMAT did not reach significance. Given the key role of serotonin signalling in the development of cortical circuitry, our data suggest that these pathways may also facilitate rTMS induced structural reorganisation of brain circuits to produce long term functional change. (247 words)

POS-THU-060

ISCHAEMIA INDUCED CELL DEATH IS SELECTIVELY MEDIATED THROUGH CaMKII PHOSPHORYLATION AT T253

Skelding KA, Hoffman A, Dickson PW, Rostas JAP
School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, and Hunter Medical Research Institute, University of Newcastle, Callaghan NSW 2308

The regulatory enzyme CaMKII (Ca^{2+}-calmodulin stimulated protein kinase II) is activated following a stroke and its activity is a major determinant of ischaemia induced neuronal cell death (1). We have shown that, following either a stroke in vivo or excitotoxic stimulation of brain slices in vitro, CaMKII autophosphorylation at T253 correlates with the degree of ischaemia-induced cell death (2). Using a human neuroblastoma (SHSY5Y) that, when differentiated *in vitro*, resembles a dopaminergic neuron, we have established a model system to test whether this correlation represents a causal link. We have established stably transfected cell lines that will inducibly express phosphomimic, phospho-null or wild type (WT) forms of αCaMKII. We have shown that these cells undergo dose and time dependent glutamate-induced excitotoxic cell death that is inhibited by the specific CaMKII inhibitor myr-AIP applied following glutamate-stimulation. When stimulated with 1mM glutamate (excitotoxic stimulus) the control cells transfected with empty vector (EV) showed ~15% loss of cell viability (resazurin assay) after 24 hrs. However, cells induced to overexpress the T253D-αCaMKII phosphomimic showed greatly enhanced loss of viability ($n=5$, $p<0.01$) which did not occur in cells overexpressing the T253V phospho-null αCaMKII. The smaller enhancement of loss of cell viability observed with WT and T286D phosphomimic αCaMKII was not statistically significant. These results support the conclusion that ischaemia induced cell death is selectively mediated through CaMKII phosphorylation at T253.

1. Vest, RS, et al. (2010) J Biol Chem 285: 20675-82.
2. Skelding KA et al (1012) *J Cereb Blood Flow Metab* 32: 2181-92.

Word count (text + refs)= 249

POS-THU-061

DEPLETION OF TRIM32 PROTECTS MICE FROM ANXIETY-AND DEPRESSION-LIKE BEHAVIORS UNDER MILD STRESS

Ruan CS[1,3], Wang SF[1], Xiao ZC[1,2], Zhou XF[1,3].

1. Key Laboratory of Stem Cell and Regenerative Medicine, Institute of Molecular and Clinical Medicine, Kunming Medical University, Kunming, Yunnan, P.R. China. 2. Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia. 3. School of Pharmacy and Medical Sciences, Faculty of Health Sciences, University of South Australia, Adelaide, South Australia, Australia.

Depression is one of common mental disorders in the adult but its mechanism is not well understood. TRIM32, an E3-ubiquitin ligase associated with the processes of apoptosis and neurogenesis, might play a role in regulating the molecular mechanism of depression. Here, we propose that TRIM32 may be involved in the development of anxiety and depressive behaviours after stress. Using a chronic unpredicted mild stress (CUMS) mouse model, we investigated the expression of TRIM32 in the brain and observed the behavioural changes in TRIM32 knockout mice. The results showed that CUMS in mice induced anxiety-like and depression-like behaviours, CUMS in mice induced significant reduction in TRIM32 protein but not its mRNA in the brain. The behavioural changes and the reduction of TRIM32 protein could be reversed by fluoxetine treatment. Finally, it is found out that mice with TRIM32 knockout showed hyperactivities and were resistant to the development of anxiety-like and depression-like behaviours triggered by CUMS stimuli. We conclude that TRIM32 plays important roles in the regulation of hyperactivities and the depletion of TRIM32 is resistant to the development of anxiety and depression mood disorders induced by chronic stress. (187 words)

POS-THU-062

MAGNESIUM-POLYETHYLENE GLYCOL AS A POTENTIAL NOVEL THERAPEUTIC APPROACH TO OEDEMA FORMATION FOLLOWING TRAUMATIC BRAIN INJURY

Diana Busingye, *Adelaide Centre for Neuroscience Research, School of Medical Sciences*
Further investigators: Renée J. Turner and Robert Vink
Adelaide Centre for Neuroscience Research, School of Medical Sciences

Background and Aims: Traumatic brain injury (TBI) is responsible for more deaths in individuals under 45 years of age than any other cause of death. Survivors are often left with life-long disabilities that contribute to the enormous socio-economic burden that TBI places on both the family and the community. Despite the significance of the problem, no effective therapy has been developed to date. A number of experimental studies have shown that decline in intracellular free magnesium is a ubiquitous feature of TBI, and that restoration of magnesium homeostasis improves both cognitive and motor outcome. However, a recent large, randomized clinical trial of magnesium in TBI failed, in part because of poor central penetration of the magnesium salt. Subsequent studies in spinal cord injury have shown that magnesium penetration into the CNS can be facilitated if the magnesium salt is administered in a solution containing polyethylene glycol (PEG), a hydrophilic polymer that facilitates transport across the blood brain barrier and throughout the extracellular space. Therefore, the present study will characterise the therapeutic potential of combined magnesium chloride and PEG in TBI, specifically examining its effect on oedema, a known secondary injury factor associated with over 50% of total TBI mortality and morbidity.

Methods: Adult male Sprague rats (360-400g) were anaesthetized with 5% Isoflurane delivered in 1.5L/min O_2 and then placed on a thermostatically controlled heating pad where 1.5-2% Isoflurane maintenance anesthesia was commenced. After injection of Lignocaine (2%) as a local anesthetic, a midline incision was made on the shaved dorsal surface of the head and the skin retracted to expose the skull. A 9 mm circular, stainless steel disc (2 mm deep) was then attached centrally to the skull over the midline using polyacrylamide adhesive and moderate injury induced using the impact acceleration model of TBI (2 metres). A 10 min hypoxic (10% O_2 in nitrogen) period was subsequently induced to replicate apnea in the ventilated animals. At 30 min after injury, animals (n=6/group) were randomly administered either 254 µmoles/kg $MgCl_2$, 254 µmoles/kg $MgCl_2$ in 1 g/kg PEG, PEG alone or equal volume saline vehicle. A separate group of surgically prepared animals were neither injured or treated and served as shams. At 5 h after TBI, the brain water content was determined by the wet weight/dry weight method using a moisture analyser.
For blood brain barrier (BBB) permeability: At 30 mins prior to death 0.4ml of 4% Evans blue was given via the tail vein. Animals were saline perfused. The brains were homogenized with phosphate buffered saline (PBS) and trichloroacetic acid (TCA) added. The EB absorbance was measured with a spectrophotometer at 620nm.

Results: Rats treated with either saline or PEG alone showed a significant increase ($p<0.05$) in brain water content relative to sham animals. Animals treated with either $MgCl_2$ or $MgCl_2$ in PEG demonstrated a 44-57% reduction in oedema compared with vehicle treated controls; both magnesium treatment groups were not significantly different from sham animals. For BBB, rats treated with either saline or PEG alone showed a significant increase ($P<0.005$) in BBB relative to the sham animals.

Conclusion: Magnesium administered after TBI either alone or in combination with PEG reduced subsequent oedema formation at 5 h posttrauma. Further studies will examine the effects of combined magnesium-PEG on blood brain barrier permeability, functional outcome and nigrostriatal neurodegeneration, with subsequent dose response studies determining whether PEG facilitates magnesium's neuroprotective effects.

POS-THU-063

MITOCHONDRIAL CONTRIBUTIONS TO NEURONAL AUTOPHAGY: LINKS TO ENERGETICS AND MITOPHAGY?

Shin YS[1], Ryall JG[2], Britto JM[1], Higgins GC[3], Nagley P[3] and Beart PM[1]

1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC 3010, Australia. 2. Department of Physiology, University of Melbourne, Victoria, 3010, Australia. 3. Department of Biochemistry and Molecular Biology, Monash University, VIC 3800, Australia.

Dysfunctional mitochondria are recognized as a common theme amongst various neuropathologies. Recent genetic studies of Parkinson's disease have revealed mutations of PINK1 and parkin, which regulate degradation of damaged mitochondria through autophagy. Here we investigated neuronal auto-/mito-phagy in primary neurons. Primary cultures of cerebellar granule cells (Swiss mice) were exposed to insults targeting mitochondrial respiratory chain complexes I-V (rotenone, 3-nitropropionic acid, antimycin A, KCN and oligomycin respectively) to induce dysfunctional mitochondria. The extent of bioenergetic failure was determined by observing the level of ATP, depolarisation of mitochondrial membrane potential and decrease in oxygen consumption rate measured by the Seahorse XF24 extracellular flux analyzer. All stressors produced mitochondrial dysfunction as shown by concentration-, time-dependent decline in ATP over 4-24h ($n \geq 3$). Neurons with dysfunctional complex I, III and IV showed rapid loss of mitochondrial membrane potential and concentration-, time-dependent decrease in oxygen consumption rate over 4-24h ($n=5$). Neurons with dysfunctional complex II showed significant reduction (~80% reduction) in mitochondrial reserve capacity and oxygen consumption, showing the most significant damage to mitochondrial bioenergetics. Investigation of autophagy was followed by observing significant accumulation of puncta acidic vacuoles in cytoplasm after 4h of drug treatment ($p<0.05$) labelled by monodansylcadaverine. Immunofluorescent detection of PINK1 antibody revealed cytoplasmic translocation of PINK1 and immunoblotting for microtubule-associated protein 1 light chain 3 (LC3-I/II) showed overall increase in LC3-II bands 24h after inhibition of respiratory complexes, especially with complex I ($p<0.05$) and II inhibition ($p<0.01$; both $n=3$), suggesting the involvement of autophagic mechanisms with likely involvement of mitophagy. (248 words)

POS-THU-064

MODELLING ALZHEIMER'S DISEASE: TRANSGENIC EXPRESSION OF HUMAN Aβ1-42 IN *CAENORHABDITIS ELEGANS* NEURONS

Sirwani NS[1], Warwick Grant[1], Gawain McColl[2]

1. La Trobe Institute for Molecular Sciences, La Trobe University, Bundoora, Australia. 2. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria 3010, Australia

Alzheimer's disease (AD), the most common reason for dementia in humans, is the third leading cause of death in Australia. One of the pathological hallmarks of AD is the formation of extracellular senile plaques in the neurons of the brain, which are composed of aggregates of fibrillar amyloid β (Aβ) peptides. Found as soluble oligomers or aggregated in insoluble plaques, the Aβ peptide is a short fragment derived from the Amyloid precursor protein by proteolytic cleavage, and varies in length ranging from 39-42 amino acids. One of the most abundant Aβ peptide species is Aβ1-42. The degree to which either insoluble or soluble species of Aβ contribute to the pathophysiology of AD remains controversial. The nematode *Caenorhabditis elegans* has been used to understand *in vivo* toxicity associated with these Aβ species, but it has been shown recently that previous transgenic strains reported in the literature express Aβ3-42 peptide (rather than the human AD relevant Aβ1-42). The aim of this project is to construct a transgenic *C. elegans* strain that expresses Aβ1-42 in the neurons, driven by the pan-neuronal *rgef-1* promoter. This strain would be validated using techniques such as mass spectrometry and immunoprecipitation and characterised phenotypically by behavioural assays to assess the extent of loss of neuronal function. This new strain(s) will provide a tool to study the molecular and cellular mechanisms of AD progression, investigate *in vivo* toxicity of Aβ, and provide the basis for *in vivo* screens to identify new therapeutics.

POS-THU-065

SYSTEMATIC REVIEW AND META-ANALYSIS OF THERAPEUTIC HYPOTHERMIA IN ANIMAL MODELS OF SPINAL CORD INJURY

Peter E Batchelor[1,2], Peta Skeers[1], Ana Antonic[1,2], Taryn E Wills[1,2], David W Howells[2], Malcolm R Macleod[3], Emily S Sena[2,3].

1. Department of Medicine, University of Melbourne, Heidelberg, Victoria, Australia. 2. Florey Institute of Neuroscience and Mental Health, Heidelberg, Victoria, Australia. 3. Division of Clinical Neurosciences, University of Edinburgh, Edinburgh, United Kingdom

Therapeutic hypothermia is a clinically useful neuroprotective therapy for cardiac arrest and neonatal hypoxic ischemic encephalopathy, and may potentially be useful for the treatment of other neurological conditions including traumatic spinal cord injury (SCI). The pre-clinical studies evaluating the effectiveness of hypothermia in acute SCI broadly utilise either systemic hypothermia or cooling regional to the site of injury. The literature has not been uniformly positive with conflicting studies of varying quality, some performed decades previously. In this study, we systematically review and meta-analyse the literature to determine the efficacy of systemic and regional hypothermia in traumatic SCI, the experimental conditions influencing this efficacy, and the influence of study quality on outcome. Our inclusion criteria consisted of the (i) reporting of efficacy of hypothermia on functional outcome (ii) number of animals and (iii) mean outcome and variance in each group.

Systemic hypothermia improved behavioural outcomes by 24.5% and a similar magnitude of improvement was seen across a number of high quality studies. The overall behavioural improvement with regional hypothermia was 26.2%, but the variance was wide. Sufficient heterogeneity was present between studies of regional hypothermia to reveal a number of factors potentially influencing efficacy, including depth and duration of hypothermia, animal species, and neurobehavioural assessment. However, these factors could reflect the influence of earlier lower quality literature.

Systemic hypothermia appears to be a promising potential method of treating acute SCI on the basis of meta-analysis of the pre-clinical literature and the results of high quality animal studies. (246 words).

POS-THU-066

INCREASED F2-ISOPROSTANES IN PARKINSON'S DISEASE HUMAN BRAIN

Abbott SK[1,2], Spiro AS [1,2], Jenner AM [1,2], Halliday GM[3], Garner B [1,2]

[1] Illawarra Health and Medical Research Institute, University of Wollongong, NSW, Australia

[2] School of Biological Sciences, University of Wollongong, NSW 2522, Australia

[3] Neuroscience Research Australia, Sydney, NSW, Australia

Our previous studies (1) revealed increased oxysterol levels in the occipital cortex (OCC) of patients with Parkinson's disease (PD). As some oxysterols (e.g. 7-ketocholesterol) are markers of lipid oxidative stress, in the present study we assessed a new cohort of control and PD samples for levels of F_2-isoprostanes as an independent marker of lipid peroxidation.

Frozen brain grey matter from 10 control cases and 9 age-matched sporadic PD cases were received from the Sydney Brain Bank and the NSW Tissue Resource Centre following study approvals. Standardised clinicopathological criteria were used for diagnosis with no case or control having any neuropathology other than PD. Lipids were extracted from frozen anterior cingulate cortex (ACC) and OCC tissue samples. F_2-isoprostane PFBenzyl esters were derivatized and analyzed using an Agilent triple quadrupole GC-MS system. Levels of F_2-isoprostane isomers (IPF_2III+VI and IPF_2IV) were calculated by comparison of specific MRM transitions with their corresponding heavy isotope internal standards.

IPF_2III+VI levels (ng/g tissue) were significantly increased in both the ACC (72%) and OCC (56%) of PD samples compared to controls (both $P<0.05$). Similarly, IPF_2-IV levels were also increased in both the ACC (68%) and OCC (82%) of PD samples compared to controls (both $P<0.05$).

These data provide further evidence for a role of lipid peroxidation in PD and also suggest that severe pathology (which is not present in either the ACC or the OCC in PD) is not a prerequisite for increased lipid peroxidation in the PD brain.

1. Cheng D et al. (2011) PLoS ONE 6(2):e17299

(241 words)

POS-THU-067

CHARACTERISATION OF TAUOPATHY IN THE PONTO-MEDULLARY BRAINSTEM NUCLEI OF AGED TAU-P301L MICE.

Stanić D, Bautista T and Dutschmann M.
Florey Institute of Neuroscience and Mental Health, Systems Neurophysiology, Parkville, Victoria, 3010.

Neurodegenerative diseases, such as Alzheimer's disease (AD) and dementia, are often linked to swallowing disorders such that aspiration pneumonia resulting from the accidental inhalation of ingested material is a common phenomenon. Growing evidence suggests that malfunction in the coordination of swallowing and breathing leads to increased risk of aspiration. This project investigates the underlying pathology linking dementia and swallowing dysfunction in Tau-P301L transgenic mice, an established mouse model of neurodegeneration. Tauopathy and neurofibrillary tangle-related neuropathological morphology in the brainstem of 8-month old Tau-P301L mice was identified by immunohistochemistry using antisera against: 1) pre-neurofibrillary tangles (Tau phosphoThreonin 231); and 2) extra-neuronal neurofibrillary tangles (Tau phosphoSerine 199/202 and AT8). Cell bodies containing diffuse phospho-tau-immunoreactivity within the cytoplasm, together with preserved, collapsed or absent dendrites were identified in the Kölliker-Fuse (KF) nucleus, ventral swallowing group (VSG) dorsomedial to the nucleus ambiguus, and nucleus of the solitary tract (NTS), regions known to co-ordinate breathing and swallowing. Other regions containing phospho-tau-immunoreactive cell bodies include the hypoglossal, facial, spinal trigeminal, cuneate, lateral reticular, medullary reticular, pontine reticular, and medial/lateral parabrachial nuclei. Our results suggest that neurons in the KF, VSG and NTS are susceptible to neurodegeneration, and provide a platform for examining the effect of tauopathy in functionally defined and experimentally controllable brainstem circuits (for swallowing and breathing). This may facilitate a framework for the development of potential drug therapies for neuroprotection and ultimately prevention of aspiration and dementia in patients suffering neurodegenerative disease. (238 words).

POS-THU-068

INVESTIGATING *SPTLC1* MUTATIONS ON PROTEIN PROFILES IN HSN-I PATIENT LYMPHOBLASTS

Stimpson S[1, 2,3,] Coorssen JR[1,2,3,4] , Myers SJ[1,2,3,4]

1. Neuro Cell Biology Laboratory. 2.Molecular Medicine Research Group. 3.School of Sciences and Health. 4.School of Medicine. University of Western Sydney, New South Wales, Australia.

Axonal degeneration is the final common path in many neurological disorders. It is seen in its pure form in hereditary axonal neuropathies. Subsets of neuropathies involving the sensory neuron are known as hereditary sensory neuropathies (HSNs). Hereditary sensory neuropathy type I (HSN-I) is the most common subtype of HSNs with autosomal dominant inheritance. It is characterized by the progressive degeneration of the DRG and an onset of clinical symptoms between the second or third decade of life. Heterozygous mutations in the serine palmitoyltransferase (SPT) long chain subunit 1 (SPTLC1) were identified as the pathogenic cause of HSN-I.

Previous studies have shown that in HSN-I patient lymphoblasts, mitochondria play a role in HSN-I. Transmission electron micrographs studies have shown that the mitochondria in the HSN-I mutant cells are morphologically challenged, cluster to the perinucleus and are wrapped by the endoplasmic reticulum (ER).

This investigation has shown that mutant SPTLC1 alters the expression of and potentially interacts with a set of proteins that associate with the mitochondria. Using mitochondrial protein isolates from control and patient lymphoblasts, via 1 and 2-dimensional gel electrophoresis, we have confirmed up regulation of two proteins; Ubiquinol Cytochrome C Reductase Core Protein 1 and Ig kappa light chain, by mass spectrometry caused by the SPTLC1 mutations.

In conclusion, previous studies has shown alteration in mitochondrial and microsomal protein profiles in patient-derived lymphoblasts, with this investigation confirming changes and has identified novel proteins which may help further the understanding of HSN-I and establish a basis for treatment. (248 words).

POS-THU-069

CELL PROLIFERATION IN ALCOHOL-RELATED BRAIN DAMAGE

Dennis CV[1], Suh LS[1], Kril JJ[1,2], Sutherland GT[1].

Disciplines of [1]Pathology and [2]Medicine, Sydney Medical School, University of Sydney, Sydney, NSW 2006, Australia.

Alcohol-related brain damage (ARBD) is a complex disorder resulting from the primary effects of alcohol on the brain in combination with liver damage and nutritional abnormalities. Hepatic encephalopathy (HE) and Wernicke's encephalopathy (WE) are potential sequelae of chronic alcoholism due to liver failure and thiamin deficiency, respectively. Animal models suggest that cell proliferation in the brain, and particularly neurogenesis, is inhibited by alcohol consumption although cell turnover recovers with abstinence. However, the role of cell proliferation in ARBD has not been previously examined. Here we report that there is no difference in cell proliferation in either the subventricular zone or subgranular zone between pathologically confirmed cases of ARBD, HE or WE and neurologically normal controls. In fact both these areas experience such a dramatic decrease in neurogenesis in early childhood that proliferative events broach the limits of detection of immunohistochemistry in the adult brain. In contrast, there is a low level of microglial proliferation in all adult brains irrespective of phenotype, with a subset of HE patients displaying extensive microgliogenesis throughout their brains. The microglia in these proliferative cases display an activated morphology while those in non-proliferative HE cases appear dystrophic. The latter cases also show greater neuronal loss in their prefrontal cortex. These findings suggest that microglial proliferation is an early neuroprotective response in HE that ultimately fails due to the continued presence of underlying etiological factors such as high cerebral ammonia and systemic inflammation (239 Words).

POS-THU-070

EARLY LIFE EXPOSURE TO LEAD AND/OR MANGANESE AND LATE LIFE NEUROBEHAVIOURAL ALTERATIONS IN RATS

Chand Basha Davuljigari, Kavitha Vonteru and Rajarami Reddy Gottipolu
Division of Neuroscience, Department of Zoology, Sri Venkateswara University, Tirupati, India. 517 502.

In the present study, we have examined the individual and combined effect of lead (Pb) and (Mn) on the synaptosomal acetylcholinesterase activity (AChE), acetylcholine (ACh), dopamine, and mitochondrial monoamine oxidase (MAO) activity in cortex, hippocampus and cerebellum regions of brain, and further evaluated the deficits in spatial learning in PND 60, 4, 12 and 18 months age groups of rats. Rats were exposed to Pb (lactationally to 0.2% Pb in drinking water of the mother), Mn (2.5mg/kg x 3 intraperitoneal injections/week) and Mn+Pb (3 intraperitoneal injections of Mn/week to Pb exposed rats) from PND 29 for period of 4 weeks. Exposure to Pb, Mn and Pb+ Mn resulted in significant decrease in the activities of AChE and MAO whereas ACh and dopamine levels were significantly increased following exposure to Pb and Mn individually and together. The effects were greater with combined (Pb+Mn) metal exposure in all age groups of rats. Mn and Pb+Mn exposed rats showed greater alterations in cortex than hippocampus and were maximum at PND 60. Significant deficits were also observed in spatial reference and working memories in the Morris water maze in all selected age groups of rats. However, these alterations were found to be maximum in older adults (12 months) and significant with Pb+Mn exposure. Thus the data demonstrate that both Pb and Mn caused alterations in neurotransmitters in age and brain region specific manner and co-exposure to Pb and Mn greatly increased the neurotoxicity of brain and behavior compared with individual metal.

POS-THU-071

INVESTIGATION OF WHITE MATTER NEURONS IN A RODENT MODEL OF MATERNAL IMMUNE ACTIVATION – IMPLICATIONS FOR SCHIZOPHRENIA

Ryan Duchatel[1-3], Phillip Jobling[1-3], Lauren Harms[2-4], Deborah Hodgson[2-4,5] and Paul Tooney[1-3,5]

1 School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, NSW, Australia; 2 Centre for Translational Neuroscience and Mental Health, University of Newcastle, NSW; 3 Hunter Medical Research Institute, Newcastle, NSW; 4 School of Psychology, Faculty of Science and IT, University of Newcastle, NSW; 5 Schizophrenia Research Institute, Sydney.

Maternal immune activation (MIA) is a risk factor for development of schizophrenia. Post-mortem studies identified increased white matter neuron (WMN) density underneath the cortex in schizophrenia. This study characterised WMNs in rat brains and investigated the effects of MIA on their density. Brains from 10-12 week old Wistar (n=2) and Sprague Dawley (n=4) rats were fixed in paraformaldehyde and processed for DAB-immunohistochemistry (DAB-IHC) or immunofluorescence using antibodies directed against NeuN, V-GLUT2 and GABAergic interneuron markers. In the MIA model, pregnant Wistar rats were injected with PolyI:C or vehicle (Sham) on gestational day 10 (GD10:PolyI:C n=6) or 19 (GD19:PolyI:C n=10, Saline n=6). Offspring were anaesthetised at 12 weeks; perfuse fixed, brains removed and processed for DAB-IHC and WMN density determined. NeuN positive (NeuN+) WMNs were observed in the corpus callosum of untreated rats; some of which co-stained for GAD65/67 or NPY. A 9% decrease in NeuN+ WMN density was observed in rostral regions of the corpus callosum in the GD10 rats whereas a 19% increase was observed in GD19 rats, compared to sham controls. Co-staining with antibodies to VGlut2 identified putative excitatory neuronal inputs on the soma and dendrites of some NeuN+ lWMNs. This study showed that: (1) a proportion of WMNs in the rat corpus callosum contain markers for GABAergic interneurons; (2) some WMNs have putative excitatory synaptic inputs; and (3) MIA at key time points in development may have contrasting affects on WMN density. Whether these findings relate to the increased density of WMNs in schizophrenia warrants further investigation.

Word count = 250

POS-THU-072

A MULTIFACETED PEPTIDE THAT BINDS MONOMERIC AND AGGREGATED BETA-AMYLOID AND INHIBITS THE FORMATION OF NEUROTOXIC OLIGOMERS.

Fraser P.E.[7], Martins, R.N[1,2,4,5].

[1]Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical Sciences, Edith Cowan University, Joondalup, WA., Australia [2]Alzhyme, Pty., [3]School of Biomedical Sciences, Curtin University, Bentley, WA, Australia [4]McCusker Alzheimer's Research Foundation, [5]School of Psychiatry and Clinical Neuroscience, UWA., Crawley WA, Australia. [6]Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Vic, Australia, [7]Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario.

Although the formation of beta-amyloid (Aβ) deposits in the brain is a hallmark of Alzheimer's Disease (AD), the soluble oligomers rather than mature amyloid fibrils contribute to Aβ toxicity and neurodegeneration. Thus, the discovery of agents targeting soluble Aβ oligomers is highly desirable for early diagnosis prior to the manifestation of a clinical AD phenotype and also more effective therapies. We have previously reported that a novel 15mer peptide, isolated via phage display screening, targeted Aβ and attenuated its neurotoxicity. Here, we aimed to generate and biochemically characterise analogues of this peptide with improved stability and therapeutic potential. We found that a stable analogue of the 15mer (15M S.A.) retained the activity and potency of the parent peptide and demonstrated improved proteolytic resistance *in vitro* (stable to t=300min c.f. t=30min for the parent peptide). This candidate reduced the formation of soluble Aβ42 oligomers, with the concurrent generation of non-toxic, insoluble aggregates measuring up to 25-30nm diameter as determined by atomic force microscopy. The 15M S.A. candidate directly interacted with oligomeric Aβ42, as shown by co-immunoprecipitation and Surface Plasmon Resonance/Biacore analysis, with an affinity in the low micromolar range. Furthermore, this peptide bound fibrillar Aβ42 and also stained plaques *ex vivo* in brain tissue from AD model mice. Given its multifaceted ability to target monomeric and aggregated Aβ42 species, this candidate holds potential for novel preclinical AD imaging and therapeutic strategies. (230 words)

POS-THU-073

GROWTH FACTORS ARE ALTERED IN NEUROGENIC REGIONS OF THE PARKINSON'S DISEASE BRAIN

Virachit S[1,2], Werry E[3], Galli E[7], Rappou E[7], Pulkkila P[7], Saarma M[7], Halliday G[1,2], Shannon Weickert C[1,5,6], Double KL[4,1,2]

[1.]Neuroscience Research Australia, Randwick [2.]Brain Sciences University of New South Wales, Sydney [3.]Faculty of Health Sciences, University of Sydney [4.]Discipline of Biomedical Science, School of Medical Science, Sydney Medical School, University of Sydney [5.]School of Psychiatry, University of New South Wales, Sydney [6.]Schizophrenia Research Institute, Darlinghurst [7.]Institute of Biotechnology, University of Helsinki, Finland.

Growth factors play a critical role in the proliferation and survival of progenitor cells in the adult brain and are proposed to regulate neurogenesis in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Proliferating cells in the hippocampus and SVZ are decreased in the Parkinson's disease (PD) brain. The current study investigated levels of growth factors including basic fibroblast growth factor (FGF2), epidermal growth factor (EGF), heparin-binding epidermal growth factor (HB-EGF), glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). We employed enzyme-linked immunoassays (ELISA) to quantify levels of growth and neurotrophic factors in fresh frozen hippocampal and SVZ tissue from 9 control and 10 age-matched PD brains. Hippocampal levels of FGF2 were significantly increased by 29%, whilst levels of GDNF were significantly decreased by 19% in the PD brain. Hippocampal levels of EGF, HB-EGF and BDNF did not differ between PD and control brains. In the SVZ, levels of EGF (30%) and GDNF (29%) were significantly decreased in PD, whilst levels of FGF2 were increased by 22% in PD. Levels of HB-EGF in the SVZ did not significantly differ between PD and control brains. Decreased levels of the potent neuronal survival factor GDNF may underlie observed decreases in neurogenesis in PD and increases in the potent mitogen FGF2 may represent a compensatory response to decreased cellular proliferation. (227 words)

POS-THU-074

TAU POSITIVE NEURONS SHOW A MARKED MITOCHONDRIAL LOSS IN ALZHEIMER'S DISEASE.

Wee Y[1] and Power J[1]

[1]School of Medicine, Flinders University, SA

Alzheimer's disease (AD) results in marked neuronal loss and the aggregation of the intracellular microtubule associated protein tau and mitochondrial dysfunction have been implicated. In this study we have used control and AD post-mortem tissue, mitochondrial and tau markers and immunohistochemical techniques to determine if mitochondrial loss is a feature of tau positive neurons. Light microscopy confirmed that our tau antibody recognised a variety of forms of tau aggregations in axons and in the neuronal cell body. Co-localisation of tau with the mitochondrial protein (superoxide dismutase 2) using confocal microscopy showed a marked decrease in mitochondria in tau positive neurons compared with tau negative neurons in the same tissue and with control tissue. Using image J to quantitate the fluorescence from ten tau positive and negative neurons from five cases of AD at Braak stage 5/6 and from four control cases we have shown that there is a significant 40% decrease in mitochondria in tau positive neurons. There were no significant differences between tau negative neurons and control tissue neurons. In addition to mitochondrial loss, tau aggregation in neurons was also associated with nuclear material that was stained with the nuclear dye Dapi. Results from this study suggest that the mitochondrial dysfunction reported to be associated with AD is more likely to be mitochondrial loss. Mitochondrial loss with a resulting decrease in ATP and decreased intracellular trafficking due to the hyperphosphorylated tau would contribute to the axonal regression and synaptic loss seen in AD.

POS-THU-075

MICROTUBULE DYNAMICS IN HEREDITARY SPASTIC PARAPLEGIA-PATIENT DERIVED OLFACTORY STEM CELLS.

Weyers S[1], Yongjun F[1], Crane D[1], Sue CM[2], Mackay-Sim A[1]

1.National Centre for Adult Stem Cell Research, Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland, 4111, 2.Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St Leonards, NSW, 2065

Hereditary Spastic Paraplegia is a genetically heterogeneous group of disorders affecting the long motor neurons of the corticospinal tract with a clinical phenotype of progressive muscle weakness and spasticity of the lower limbs. Patients with mutations in *SPAST*, the gene for the microtubule severing protein spastin, can present a phenotype indistinguishable from idiopathic cases for which causal genetic mutations are not known. We have established a stem cell model for HSP using olfactory neurosphere-derived (ONS) cells from HSP patients and healthy controls. Patient-derived cells show disease-associated differences in microtubule-associated gene expression and altered expression of microtubule-associated proteins including acetylated alpha-tubulin, indicating significant down-regulation of stable microtubules. We investigated the dynamics of microtubule formation in ONS cells from *SPAST* patients and compared them with cells from non-*SPAST* patients with similar clinical phenotype. To investigate the dynamics of microtubule formation we used a fluorescently tagged tubulin end-binding protein and quantitated microtubule dynamics using time-lapse imaging and automated image analysis. In ONS cells from *SPAST* and non-*SPAST* patients there was a significant reduction in density of newly forming microtubules compared to healthy controls. Differentiation of ONS cells into neuron-like cells increased the patient-control difference in density of newly forming microtubules and revealed an increase in the rate of microtubule formation in patient cells. These results support the hypothesis that similar clinical features in patients may result from similar cellular dysfunctions because non-*SPAST* cases had microtubule-associated dysfunctions similar to *SPAST* cases with known mutation in a microtubule-associated gene. (244 words)

POS-THU-076

A NOVEL NEUROTROPHIN-BASED STRATEGY FOR TREATING DEMYELINATING DISEASES

Murray SS[1], Wong AW[1], Tran G[2], Hodgkinson S[2], Wood R[1], Hughes RA[1], Giuffrida L[1], Kilpatrick TJ[1] and Xiao J[1].

[1]The University of Melbourne, Victoria; [2]The University of New South Wales, NSW, Australia

Abstract

Developing novel neurotrophin-based therapeutic strategies could promote repair in a number of neurodegenerative diseases. We have identified that BDNF promotes peripheral nervous system myelination via $p75^{NTR}$. We have generated cyclo-DPAKKR, a small peptide that structurally mimics the region of BDNF that binds $p75^{NTR}$, and shown that it selectively targets $p75^{NTR}$ to uniformly promote peripheral myelination during development. Here we aim to investigate whether cyclo-DPAKKR can reduces myelin loss and promote myelin repair in experimental autoimmune neuritis (EAN), a rodent model of peripheral demyelinating neuropathy. Cyclo-DPAKKR administration significantly reduced the clinical disease severity in EAN (n=12), significantly abrogated the reduction in myelin protein expression, and significantly reduced the loss of myelinated axons in EAN-induced mice compared to vehicle controls (n=4). This suggested that cyclo-DPAKKR reduces the extent of demyelination against EAN. Furthermore, expression of APP+ (a marker for acute axonal damage) was also reduced in the peripheral nerves of cyclo-DPAKKR-treated animals (n=4), suggesting that cyclo-DPAKKR not only reduces the extent of demyelination but also inhibits axonal damage in EAN. In addition, EAN animals that were continuously administrated cyclo-DPAKKR after disease peak exhibited significantly faster recovery compared to controls (n=6), suggesting that cyclo-DPAKKR also promotes repair following EAN. Collectively our data demonstrate that cyclo-DPAKKR ameliorates peripheral demyelinating neuropathy by protecting against demyelination and by inhibiting axonal damage. Our findings suggest that selective targeting of $p75^{NTR}$ is potentially novel therapeutic strategy to treat human demyelinating peripheral neuropathies.

POS-THU-077

MINOCYCLINE HAS LIMITED EFFECTS ON EARLY MICROGLIAL ACTIVATION FOLLOWING PHOTOTHROMBOTIC STROKE IN RATS

Yew WP, Jayaseelan JSP, Djukic ND, Muyderman H, Sims NR
Centre for Neuroscience and Discipline of Medical Biochemistry, School of Medicine, Flinders University, Adelaide, S.A., Australia

Treatment with the antibiotic, minocycline, in animal models of stroke has been found both to reduce tissue damage and improve functional recovery. Anti-inflammatory effects of minocycline, including its ability under some conditions to inhibit the activation of microglia, are commonly proposed to contribute to these positive effects. However, evidence for direct action of minocycline on microglia in the brain has only occasionally been presented. To address this issue, we tested the effects of minocycline on microglial activation following induction of a stroke by photothrombosis in the forelimb region of rat motor cortex. Minocycline (50 mg/kg) or vehicle was injected intraperitoneally at 1 and 24 hours after stroke. This protocol essentially mimicked the initial phase of treatment in many of the previous studies that showed positive effects of minocycline. Microglial activation was assessed using methods we have developed to detect changes in cell morphology and distribution in the peri-infarct tissue. Marked alterations in both properties were detected between 1 and 7 days after stroke in both treatment groups. Compared with vehicle treatment, minocycline produced a moderate reduction in microglial density on days 3 and 7 in a narrow band of tissue close to the lesion boundary. No significant effects on microglial morphology were detected. These studies provide evidence that moderately high doses of minocycline have only a limited ability to modify the activation of microglia induced by photothrombotic stroke and suggest that direct effects on microglia may not be a major contributor to the positive effects of minocycline in other studies.

POS-THU-078

CHARACTERING NOVEL ALTERNATIVELY SPLICED ISOFORMS OF THE EPHA4 GENE.

Zhao J1, Narayanan RK1, Mangelsdorf M1, Boyd AW2, Bartlett PF1.

1.Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia.
2.Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Alternative splicing (AS) is a common and essential phenomenon in gene regulation, and disruption of AS is associated with human genetic diseases. There are currently >20 genes in which mutations cause amyotrophic lateral sclerosis (ALS), and a growing number of these play a role in RNA metabolism, including pre-mRNA splicing. We have identified *EPHA4* as one gene whose pre-mRNA splicing is regulated by TDP-43. Given that *EPHA4* has been implicated as a modifier of ALS, it is of benefit to characterise alternative isoforms of *EPHA4* and what role they play in ALS. Expressed sequence tags (ESTs) from the UniGene clusters of both mouse and human *EPHA4* were searched and revealed the presence of possible novel isoforms. Three of these novel isoforms were found in both human and mouse brain and spinal cord. To determine if these isoforms encode protein we performed Western blot hybridization using antibodies against both the N- and C-terminus of EphA4. This revealed several protein bands, two of which were consistent with predicted protein sizes of the novel mRNA isoforms. The function of these novel isoforms is being investigated as they may be important in mediating the progress of ALS and thus be targets for disease therapy. (200 words).

POS-THU-079

ALZHEIMER'S DISEASE-RELATED LYSOSOMAL DYSFUNCTION DISRUPTS COBALAMIN TRANSPORT IN VITRO AND IN VIVO

H Zhao [1,2], Hongyun Li[1,2], K Ruberu[1,2], B Garner [1,2]

[1] Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW, Australia [2] School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia.

Lysosomes become dysfunctional in Alzheimer's disease (AD) neurons and in aged post-mitotic cells and this may impair intracellular cobalamin (Cbl) transport. To provide a detailed analysis of the impact that physiologically relevant impairments of lysosomal function have on intracellular Cbl transport, cells were treated with lysosome-modifying compounds and assessed to investigate alterations of [^{57}Co]Cbl trafficking in subcellular compartments. Experiments were also conducted in wild-type (WT) and APPxPS1 AD mice *in vivo*. Human fibroblasts and neurons were treated with either chloroquine (to increase lysosomal pH) or leupeptin (to inhibit lysosomal proteases) and incubated with [^{57}Co]cyanoCbl. Cells were lysed and fractionated and [^{57}Co] radioactivity was measured in lysosomal, mitochondrial and cytosolic fractions. The results showed that fibroblast and neuron lysosomal [57Co]Cbl levels were increased two-fold and ten-fold after chloroquine, and three-fold and ten-fold after leupeptin treatment, respectively (all $P<0.05$). APPxPS1 mouse brain lysosomal [57Co]Cbl levels were also increased by 56% compared to the WT mouse brain levels ($P<0.01$). The *in vivo* experiments were mimicked using SH-SY5Y/APP cells that were treated with MG-115 to induce lysosomal amyloid-β (Aβ) accumulation and this similarly increased lysosomal [57Co]Cbl levels by 85% ($P<0.01$). These findings indicate that pathologically relevant processes inhibit lysosomal function and subsequently impair intracellular Cbl trafficking. The study provides the first evidence that lysosomal Cbl intracellular transport is impaired in an AD mouse model and suggests a potential therapeutic target in human AD. (229 words)
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POS-THU-080

SODIUM SELENATE REDUCES NEURODEGENERATION AND SPONTANEOUS SEIZURES IN A POST- STATUS EPILEPTICUS RAT MODEL OF TEMPORAL LOBE EPILEPSY

Zheng P1, Shultz, SR1, Wright D2, Johnston L2, Hovens C3, Jones N1, O'Brien TJ1

1. Department of medicine, Royal Melbourne Hospital, University of Melbourne
2. Department of Radiology, Florey Neuroscience Institute
3. Department of Surgery, Royal Melbourne Hospital, University of Melbourne Email: zhp@student.unimelb.edu.au

Hyperphosphorylated tau (h-tau) has been implicated in many neurological diseases, including epilepsy, neurodegeneration and psychiatric disorders. In this study, we investigated whether treatment with sodium selenate, a drug that reduces the pathological hyperphosphorylation of tau by increasing protein phosphatase 2A (PP2A) activity, would reduce neurodegeneration and spontaneous seizures in a rat model of epilepsy. After four hours of status epilepticus induced by systemic kainic acid (KA) injections, or control-saline injections, young-adult male Wistar rats (n=9 /group) were given continuous sodium selenate treatment (1 mg/kg/day), with a subcutaneous osmotic mini-pump for two months, followed by two-week drug washout. In-vivo MRI imaging was used to assess the structural damage at one month post-injury. Video-EEG recording was used to evaluate the seizure frequency and duration. Selenate treatment significantly reduced seizure frequencies in KA rats during the treatment phase (0.1 seizures /day vs. 1.4 seizures /day, $P<0.05$), and this effect was maintained after two-week washout (2.6 seizures /day vs. 8.6 seizures /day, $P<0.05$). Furthermore, in the KA+saline rats, there were enlarged lateral ventricles and shrinkage of hippocampus. In contrast, selenate treatment could reverse these pathological changes. Molecular analysis revealed that selenate treatment was associated with increased activity of PP2A and decreased expression of phosphorylated tau in the hippocampus and cortex. We concluded that sodium selenate treatment had disease-modifying effects in the rat model of temporal lobe epilepsy, with antiepileptogenic effects against seizures and mitigation of neurodegeneration. The phosphorylated tau and PP2A may involve epileptogenic modifications. (237 words)

POS-THU-081

INTERACTION BETWEEN ABETA, P75NTR AND APP FORMS A POSITIVE FEED- FORWARD LOOP PROMOTING AMYLOID BETA GENERATION

Zhou XF1, Saadipour K1,2, Smith K2, Yang M.1 Wang YR1,3, Zhong JH1, Wang YJ3

1. School of Pharmacy and Medical Sciences, Sansom Institute, University of South Australia.

2. Department of Human Physiology, Flinders University; 3. Department of Neurology, Daping Hospital, Chongqing，China.

P75NTR is upregulated during aging and in Alzheimer's brains but its role in the development of Alzheimer's disease (AD) is not fully understood. In the present study, we have investigated potential roles of p75NTR in Aβ production *in vitro* and *in vivo*. The interaction between p75NTR and APP was demonstrated by co-immunoprecipitation and FRET analysis. P75NTR ligands Aβ and pro-neurotrophins increased the FRET signal between p75NTR and APP in a dose and time dependent manner. Blocking endogenous Aβ with 6E-10 antibody reduced the FRET signal. Sequence mapping indicated Aβ interacted with cysteine rich domain (CRD) 2 and 4 but not 1 and 3 of the p75NTR ectodomain. Aβ25- 35 increased sAPPβ fragment and Aβ production by cortical neurons in AD mice but not in AD mice with the deletion of p75NTR. Transfection of p75NTR in CHO-APP695 cells increased the beta site cleavage of APP and production of Aβ. Furthermore, the FRET data showed that Aβ and proNGF increased the interaction of APP and BACE1 in a p75NTR dependent manner. Finally, we found that Aβ increased the expression of both APP and BACE1 in cortical neurons of wild type mice but not p75NTR KO mice. Our data indicates that Aβ and pro-neurotrophins promote p75NTR-APP interaction and form a positive feed- forward loop promoting beta cleavage of APP and Aβ production.

POS-THU-082

BRAINSTEM CHANGES AFFECTED BY 670 NM PHOTOBIOMODULATION TREATMENT FOLLOWING T10 HEMI-CONTUSION SPINAL CORD INJURY

Zhu S, Hu D, and Potas JR
Eccles Institute of Neuroscience, The Australian National University, Canberra, Australia

Purpose: We previously demonstrated that photobiomodulation (PBM) treatment with 670 nm light alters macrophage polarisation, reduces cell death and promotes axonal regrowth in the spinal cord following a T10 hemi-contusion spinal cord injury (T10-hSCI). Here, we examined the effects of PBM on brainstem regions following T10-hSCI as well as the development of allodynia. Methods: We induced a T10-hSCI by weight-drop (10 g, 25-50 mm), and half the cohort treated daily for 30 min using 670 nm light. All subjects underwent behavioural locomotor and sensory testing during recovery. Electrophysiology was used to assess sensory recovery of dorsal column pathways, and a modified von Frey hair test used to evaluate signs of pain in response to innocuous stimulation of the dorsum over 6 regions. Brainstems were collected at 3, 7 and 14 days post-injury, and the dorsal column nuclei (DCN) and ventral lateral tract (VLT) regions were investigated using TUNEL to quantify cell death, and immunohistochemistry to quantify activated microglia/macrophage (ED1) and axonal spouting (GAP43). Results: TUNEL-positive and ED1-positive cell numbers were significantly reduced in the VLT region at 14 days in the group that received treatment. GAP43 was significantly elevated in both regions in 7d treated animals. Electrophysiology revealed improved conduction through the dorsal columns in PBM treated animals at 7d, which also presented with reduced incidence and severity of signs of allodynia. Conclusion: These findings demonstrate that 670 nm PBM treatment can induce favourable changes up stream to the spinal level of injury. (243 words)

POS-THU-083

ITSN1 AND CELL SIGNALLING PATHWAYS

MALAKOOTI N[1], PRITCHARD MA[2], ADLARD PA[1], FINKELSTEIN DI[1]

1. The Florey, the University of Melbourne, Parkville, Victoria, Australia 2. Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

The intersectin-1 (ITSN1) gene is located on human chromosome 21 and over-expressed in Down syndrome. ITSN1 is a scaffolding protein and is involved in endocytosis and exocytosis which is thought to contribute to pre- and post-synaptic plasticity. Studies suggest that MAPK signalling has a fundamental role in learning and memory consolidation. In this study we examined cell signalling pathways. Specifically, the MAPK signalling pathways by examining the basal levels of activated ERK1/2 protein in both hippocampal homogenates of ITSN1-KO and WT mice and in hippocampal neuronal cell culture after induction of chemical-LTP.

Protein lysates were generated from the hippocampus of adult mice (9-12 w) from ITSN1-KO and WT groups. Western blot were performed. Hippocampal neuronal cell cultures derived from KO and WT were treated with or without glycine (200 µM) for 3 minutes to induce “chemical” long term potentiation. Cells were examined at different time points (0-40 minutes) post-stimulation, cells were harvested, then prepared as whole cell homogenates for western immunoblotting.

In adult mice hippocampus, levels of activated ERK1/2 in KO samples were significantly lower than in the WT ($p^*=0.0413$, $n=6$). Results for hippocampal cell culture indicated that both groups had the same pattern of activity for ERK1/2. ERK1/2 activity dropped to zero at 1 minute. After 5 minutes activity increased in both groups with no differences between the genotypes.

These data indicates that the ITSN1 gene has a role in basal MAPK signalling pathways. Hence when over-expressed, ITSN1 may contribute to the learning and memory deficits characteristic of DS. (249 words)

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POS-THU-084

THE ROLE OF PREFRONTAL CORTEX IN CONFLICT-INDUCED BEHAVIOURAL ADJUSTMENT.

Mansouri FA1,2, Buckley MJ3, Tanaka K2.

1- Department of Physiology, Monash University, VIC, Australia.
2- Cognitive Brain Mapping Laboratory, RIKEN Brain Science Institute, Wako, JAPAN.
3- Department of Experimental Psychology, Oxford University, Oxford, UK.

The context-dependent tuning of executive control optimizes the usage of our limited cognitive resources to perform prioritized tasks. After experiencing competition/conflict between behavioural choices, our behaviour is adaptively modified for a better resolution of the conflict. Studying the neural substrate and mechanisms of conflict-induced behavioural adjustment has opened a window to the neural basis of executive control. To determine the functional role of different lateral, orbital and medial frontal areas in conflict-induced executive control adjustment, we conducted complementary lesion and single-cell recording studies with monkeys. The monkeys were trained to perform a matching task in which they had to resolve a conflict between two abstract rules. We found striking similarities in conflict-induced behavioural adjustment between humans and monkeys providing a model to study the underlying neural substrates and mechanisms. The conflict-induced behavioural adjustment persisted after bilateral lesion within anterior cingulate cortex (ACC) or within dorsomedial prefrontal cortex (DMPFC), but disappeared following lesions in the principal sulcus within dorsolateral prefrontal cortex (DLPFC) or in the orbitofrontal cortex (OFC). Our recording studies showed that neuronal activities in DLPFC and OFC encoded the current conflict level suggesting that both DLPFC and OFC are involved in extracting and representing conflict. In addition, in OFC, neuronal responses to reward were modulated by the conflict experienced in the course of achieving the reward suggesting that OFC is involved in integrating conflict and reward information. These results suggest that DLPFC and OFC, but not ACC or DMPFC, are essential for the conflict-induced executive control adjustment. (248 words).

POS-THU-085

ENTRAINMENT IN RHYTHMICAL COORDINATION BETWEEN WHOLE-BODY MOVEMENT AND SELF-VOCALIZATION

Miyata K, Kudo K.
Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

Studies of rhythmic coordination have reported that the particular phase of human rhythmic movements can be entrained to external auditory pacing signal in sensorimotor synchronization (SMS) tasks. While various movements are synchronized to external information in typical SMS tasks, sensory information can be produced without external environmental information. Using self-produced information instead of external information, in principle, a variety of rhythmic movement can be generated through sensorimotor coordination. Therefore, we examined the coordination of self-produced sensory information and rhythmic movement. In the present study, we investigated whether entrainment occurs between the information of one's own making, self-vocalization and whole-body rhythmic movement. The voice rate ranged from 80 to 180 beats per minutes (bpm) in steps of 50 bpm. Whole-body rhythmic movements were two kinds of knee bending movement: knee-flexion-on-the-voice and knee-extension-on-the-voice. In order to quantify the relationship between the movement and the voice, the phase angle of the voice time on the phase plane was calculated. Analyses of phase relation between movement and self-vocal revealed several distinct differences between conditions. Under the knee-extension-on-the-voice condition, deviation from intended phase relation at higher beat rates and enhanced fluctuations were observed. These findings indicate that auditory-motor entrainment occurs regardless of the sources of auditory information (i.e., external or internal), suggesting that the neural pathway underlying the entrainment between rhythmic movement and external environmental information can also be activated by self-generated information. (228 words).

POS-THU-086

CHRONIC ADMINISTRATION OF METHYLPHENIDATE IN RATS REDUCES LONG-TERM POTENTIATION IN THE HIPPOCAMPUS, BUT FACILITATES IT IN THE PREFRONTAL CORTEX

Morales B.[1], Carreño M.[1], Contreras D.[1], Hernandez A.[1], Burgos H[2,3], Zeise M [2]
[1]Depto. de Biología, Fac. Química y Biología, Universidad de Santiago de Chile, [2]Escuela de Psicología, Fac de Humanidades, Universidad de Santiago de Chile, [3]Instituto Internacional para el Desarrollo Cognitivo, Universidad Central de Chile

Methylphenidate (MPH) is a psychostimulant used widely in the therapy of the Attention Deficit/Hyperactivity Disorder. We investigated chronic effects of MPH on visuo-spatial learning and long term potentiation (LTP) in hippocampus and prefrontal cortex (PFC). In hippocampus study, 3 weeks old Sprague-Dawley rats were injected MPH for two weeks with 0.3, 1, 3, and 10 mg/kg and trained in a Morris Water Maze. LTP was induced and recorded in CA1 by applying a TBS at the Schaeffer collaterals of hippocampus slices prepared from same rats. *In vivo* induction of LTP in the PFC was achieved into the commissural fiber and recording at a symmetrical position superficially from the contralateral hemisphere. No change in visuo-spatial learning was observed with 0.3 up to 3 mg/kg MPH. At 10 mg/kg motor behavior was altered. In slices of rats treated with 3 and 10 mg/Kg the magnitude of TBS-dependent LTP was significantly reduced from 152.8±4.4% (control) to 119.4±13.8% and 91.8±5.4%, respectively ($n=5,7$;$^*p<0.05$). In PFC study, rats were injected 0.2, 1 and 5 mg/kg and trained in an eight arms radial maze. In the acquisition phase the rats injected 1 mg/Kg were faster and the ones receiving 5 mg/Kg slower than controls in learning to fulfill the task. Interestingly, a significant increase of the LTP *in vivo* was observed to 1 and 5 mg/Kg. These results suggest while MPH, cause only subtle differences in visual orientation tasks, It decreased LTP in the hippocampus in vitro, but increases LTP in vivo in the PFC.

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POS-THU-087

EFFECTS OF LIMITED NESTING MATERIAL ON MATERNAL BEHAVIOUR.

Maniam J[1], Antoniadis C[1], Morris MJ[1].

1. Pharmacology, School of Medical Sciences, UNSW, NSW 2052 Australia.

Adverse events early in life such as abuse or neglect increase the risk of long-term behavioural deficits, and for decades this has been modeled in rodents using postnatal maternal separation. Issues with appropriate controls, however, have led to a move to alternatives such as limited nesting material (LN). Few studies have addressed effects of LN on maternal behavior (MB). We are the first to demonstrate LN effects on MB and its interaction with subsequent maternal metabolic profile.

Sprague Dawley rat litters were subjected to normal care or LN from days 2-9 (n=7,7). MB was examined during lactation, and anxiety-like behaviours were measured 4 days postweaning. The quality of maternal care was profoundly influenced by LN, with fragmented nursing and increased frequency away from pups. LN dams showed increased anxiety-like behavior on EPM (31.3±2.1 vs 42.8±3.3% entries into open arm, $p=0.012$, $t=2.96$), and exhibited 14% lower rearing frequency compared to control dams, $p<0.01$. One month postweaning, LN dams were 12% lighter and exhibited metabolic derangements with a trend of increased plasma glucose ($p=0.08$), 50% lower fasting insulin ($p=0.01$), and reduced plasma triglycerides ($p<0.05$), suggesting a possible interaction between postpartum stress and metabolic risk.

Our results highlight a critical role for the early postpartum window in establishing normal MB and subsequent risk for metabolic deficits. We are examining hypothalamus, hippocampal and muscle gene expression to determine whether stress-related, neuro-inflammatory and neurotrophic markers associate with the behavioural and metabolic phenotype. The data will provide targets for interventions to improve postpartum health outcomes.

POS-THU-088

MATERNAL OBESITY: EFFECT OF EXERCISE DURING PREGNANCY ON BEHAVIOUR AND HIPPOCAMPAL GENE EXPRESSION IN MALE RAT OFFSPRING

Raipuria M, Bahari H, Maniam J and Morris MJ
Department of Pharmacology, School of Medical Science, University of New South Wales, Sydney, Australia

Maternal obesity (MO) is associated with multiple metabolic changes which may pre-program offspring to develop metabolic disease later in life. Effects of MO on the brain development and behaviour of offspring are less explored. Physical exercise is known to influence cognitive and emotional processes, so the present study explored the effects of voluntary exercise by lean and obese mothers during pregnancy on brain and anxiety-related behaviours in offspring. Female Sprague-Dawley rats were fed chow (C) or western high fat diet (F) and half of each group underwent voluntary exercise (E), (CE or FE) with a running wheel during gestation while the rest remained sedentary (S), (CS or FS). At 5-6 weeks, male pups were tested on the Elevated Plus Maze (EPM) and their hippocampus mRNA expression of inflammatory and FTO genes were examined at 13 weeks. Maternal obesity had no effect on anxiety-like behaviour while pups from CE mothers had increased anxiety-like behaviour. Thus pups from CE had fewer entries and spent significantly less time in the open arm of the EPM (CE 12.9±1.9 vs CS 17.8±1.3 entries and CE 45.5±7.5 vs CS 116.3±10.3 sec, $P<0.05$). Hippocampal mRNA expression of TLR-4, FTO and IL-1β were upregulated by MO ($P<0.05$), and normalised by exercise. Anxiogenic effects of maternal exercise in offspring of lean dams were associated with a low level of BDNF mRNA in the hippocampus. The issue of whether metabolic or environmental factors are associated with increased anxiety and decreased BDNF in offspring from lean exercised mothers deserves further investigation.

Words: 257
Characters: 11372

POS-THU-089

EFFECTS OF A *CLOCK* MUTATION ON COCAINE-INDUCED LOCOMOTION AND COCAINE SELF-ADMINISTRATION IN MICE

Murakami G[1], Edamura M[1], Meng H[1], Okamura H[2], Nakahara D[1]

1. Department of Psychology and Behavioral Neuroscience, Hamamatsu University School of Medicine, Hamamatsu, Japan, 2. Department of System Biology, Kyoto University Graduate School of Pharmaceutical Science, Kyoto, Japan

Few studies have examined how circadian clock genes regulate diurnal variations in the abuse-related effects of cocaine in laboratory animals. To address this question, we examined the effects of a *Clock* mutation on cocaine-induced locomotor activity and cocaine self-administration and its relationship to diurnal patterns in these behaviors. We examined a *Clock* mutation because *Clock* is a key circadian clock gene. To analyze diurnal patterns of cocaine self-administration related to a *Clock* mutation, we used a novel 24-hr continuous drug access we have developed. We found that wild-type and *Clock*-mutant mice readily acquired cocaine self-administration and showed dose-dependent cocaine-induced locomotor activity in both the light and dark phases. Reflecting the previously reported diurnal pattern of cocaine intake, cocaine self-administration was higher in the dark phase compared to the light phase. In the dark phase, cocaine-induced hyperlocomotion and the number of cocaine infusions obtained in self-administration did not differ between the genotypes. In contrast, in the light phase, *Clock* mutants showed significantly greater cocaine-induced hyperlocomotion and took more cocaine infusions than in wild-type controls. Interestingly, consistent with impulsive behavior, *Clock* mutants exhibited sporadic bouts of vastly increased responding, which did not occur in any wild-type control subject. These results indicate that a *Clock* mutation enhances cocaine-induced locomotion and self-administration during the light phase of the circadian cycle and increases impulsive responding during cocaine self-administration.

POS-THU-090

EFFECT OF HIGH FAT DIET CONSUMPTION ON MOTIVATION, PAVLOVIAN AND INSTRUMENTAL CONDITIONING IN RATS

Nguyen JCD[1], Killcross AS[2], Jenkins TA[1].

[1] School of Medical Sciences, RMIT University, Bundoora, Victoria, 3083
[2] School of Psychology, University of New South Wales, Kensington, NSW, 2052

Animal studies have consistently shown that consumption of a high fat diet can lead to impaired cognitive function, specifically spatial memory, rule learning ability and working memory deficits. The current study aimed to determine the effect of high fat diet consumption on Pavlovian Instrumental Transfer, a behavioural test used to assess the impact of a reward associated cues to induce a powerful motivational influence resulting in increased instrumental responding.

Rats were fed either a 22% fat diet (n = 16) or a control diet (n = 16) for 10 weeks, and subsequently received both Pavlovian training, in which two auditory stimuli each predicted the delivery of a distinct reward, and instrumental training, where a specific lever press earned one of the rewards used in Pavlovian conditioning. Finally, the effects of the two auditory stimuli on lever press performance were assessed in extinction.

The consumption of a high fat diet does not affect learning acquisition of both Pavlovian and instrumental conditioning ($F < 1$). In extinction, there was no general or selective motivational influence on lever press responsiveness observed in the high fat fed animals ($F_{(1,87)} = 3.793$, $p = 0.0547$). Magazine entries were found to be decreased in high fat fed animals when compared to control with a group effect of $F_{(1,116)} = 10.36$, $p < 0.05$. These results suggest that high fat fed rats may have a motivational deficit in that they will not work as hard for a reward as control rats. (244 words)

POS-THU-091

ENVIRONMENTAL ENRICHMENT INFLUENCES BOTH DEVELOPMENT AND FUNCTION OF STRIATAL CIRCUITRY WITHIN THE MOUSE

O'Connor AM, Leamey CA, Sawatari A

Bosch Institute and School of Medical Sciences, University of Sydney, Sydney, NSW, 2006, Australia

The critical period is the time of peak plasticity within the nervous system. Parvalbumin-positive (PV+) inhibitory interneurons are thought to be vital in regulating the timing of this important developmental epoch. Raising animals in enriched environments has previously been shown to affect the timing of cortical critical periods. In order to determine whether environmental enrichment similarly influenced the time course of a putative 'critical period' in the striatum, PV+ expression, along with Brain Derived Neurotrophic Factor (BDNF) levels, a driver of inhibitory interneuron maturation, were examined in animals raised in enriched (EE) versus standard (SE) housing conditions across early postnatal development. In EE animals, striatal PV+ inhibitory interneurons exhibited larger mean soma sizes at P10 ($p=0.015$, univariate ANOVA), and greater density at P15 ($p=0.005$, univariate ANOVA). BDNF levels were increased in EE animals as well ($p=0.039$, univariate ANOVA), with P10 levels approaching ($p=0.058$, univariate ANOVA) and P21 levels reaching significance ($p=0.011$, univariate ANOVA). In order to further assess whether these changes led to any long term effects in behaviours dependent on striatal function, adult animals were subjected to a test of goal-seeking behaviour known as the "puzzle-box". EE mice solved the task significantly faster than animals raised in standard environments ($p=0.008$, repeated measures ANOVA, enriched $n=21$, standard $n=18$). Our results highlight the influence of environmental factors on the development of PV+ neurons within the striatum, and further suggest that these changes induced by environmental enrichment contribute to improved cognitive performance in adult animals.

(243 words)

POS-THU-092

AMPHETAMINE ALTERS THE CHOICE PREFERENCES OF DVD-DEFICIENT MALE RATS ON A RODENT GAMBLING TASK

Peak JN[1], Turner, KM[2] and Burne THJ[2,3]

1. School of Biomedical Sciences, The University of Queensland, St Lucia, Australia
2. Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia
3. Queensland Centre for Mental Health Research, Wacol, Australia

Developmental vitamin D (DVD) deficiency is a plausible risk factor for schizophrenia, and is associated with enhanced locomotor sensitivity to psychomimetic drugs and deficits in response inhibition. Amphetamine is known to alter risk-based decision-making in male rats on the rodent gambling task (rGT). The aim of this study was to examine risk-based decision-making and the effect of amphetamine in male and female DVD-deficient and control rats on the rGT.

Adult male and female Sprague-Dawley rats from control and vitamin D deficient dams (n=10-14/sex/diet) were trained to perform the rGT in operant chambers and were challenged with dex-amphetamine (0.75 and 1.5mg/kg, i.p.), with two saline injections between doses.

DVD-deficient and control rats showed significant preference for the optimal choice-option ($F_{3,135}$=16.01, p<0.001) in the rGT. Dex-amphetamine increased premature responding (t_{24}= 3.31, p=0.003) at 0.75mg/kg, and dose-dependently decreased pellets earned (F=68.87, p<0.001) and trials completed (F=68.31, p<0.001). Amphetamine-induced premature responding was accentuated in DVD-deficient rats (saline vs 0.75mg/kg, t_{10}=-2.89, p=0.016; n=7/group), and amphetamine significantly altered choice behaviour in DVD-deficient rats evident as a sub-optimal shift in preference (n=11) (0.75mg/kg, $F_{3,30}$=6.56, p=0.015).

DVD-deficient and control rats developed a preference for the optimal option in the rGT. Amphetamine increased impulsivity in DVD-deficient rats performing this task and impaired overall performance in control and DVD-deficient rats. The rGT is based upon the IOWA gambling task, in which schizophrenia patients show impaired performance. These results show that DVD-deficient rats are sensitive to amphetamine-induced disruption of optimal choice-behaviour and this may be informative for modelling schizophrenia. (250 words).

POS-THU-093

PARTICIPATION OF THE L-ARGININE-NITRIC OXIDE-cGMP-PKC-K^+_{ATP} CHANNEL SIGNALLING PATHWAY IN ZERUMBONE-INDUCED ANTINOCICEPTION

Enoch KP[1], Sulaiman MR[1], Mohamad AS[1], Akira A[1], Israf DA[1] and Hemabarathy B[2]

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia.
[2]Department of Biomedical Sciences, School of Diagnostic and Applied Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

Currently available drugs used to manage pain produce various adverse effects. In addition, chronic pain does not effectively respond to the multimodal approach of conventional therapy, thereby requiring alternative remedies. *Zingiber zerumbet*, a widely cultivated ginger species in Asia is known to be traditionally used for cuts and sores. To investigate its traditional use, we recently isolated zerumbone, a major compound from the rhizome of this plant. Our preliminary findings show zerumbone possesses potent analgesic properties. The present study investigated the participation of nitric oxide (NO), cyclic guanosine monophosphate (cGMP), protein kinase C (PKC) and ATP sensitive potassium channel (K^+_{ATP}) pathway in antinociception produced by zerumbone using animal models of pain. Zerumbone (0.1, 1, 5, 10 and 50 mg/kg, i.p.) produced significant dose-dependent antinociception when assessed using the acetic acid-induced abdominal writhing test in mice. Likewise, zerumbone at similar dosage exhibited significant inhibition of neurogenic pain induced by the intra-planter injection of glutamate and phorbol myristate acetate (PMA, a protein kinase C activator) through the paw licking test. The pain relieving effects of zerumbone was significantly reversed by the i.p. administration of L-arginine (a nitric oxide precursor) and glibenclamide (ATP-sensitive K^+ channel inhibitor) in the writhing test. Additionally, pre-treatment with methylene blue, (a non-specific guanylyl cyclase inhibitor) enhanced the antinociceptive effects of zerumbone in the same model. Collectively, the findings demonstrate that zerumbone produces pronounced antinociception against chemical and neurogenic models of pain and suggest that the zerumbone-induced analgesia seem to involve the L-Arginine-Nitric Oxide-cGMP-PKC- K^+_{ATP} channel pathway. (248 words).

POS-THU-095

DIETARY INDUCED OBESITY DISRUPTS HIPPOCAMPALLY ENCODED TRACE FEAR MEMORIES

Reichelt AC [1,2], Westbrook RF [2] and Morris MJ [1]

1. School of Medical Sciences, The University of New South Wales, Sydney. 2. School of Psychology, The University of New South Wales, Sydney

Over-consumption of palatable high fat, high energy diets is not only contributing to the increasing obesity epidemic worldwide, but the neurophysiological effects of these diets are also proposed to impact upon cognition. Obesity and over-consumption of high fat, high energy diet have been shown to induce deficits in executive function, attention, learning and memory. Adult male Sprague-Dawley rats fed standard lab chow (N=13) or lab chow supplemented with a range of palatable high fat / high calorie foods ('cafeteria' N=14) for 9 weeks. Cafeteria diet fed rats were significantly greater in weight than chow controls ($P<0.0001$) and had increased white adipose tissue deposits ($P<0.0001$). Cafeteria diet fed rats exhibited deficits in a trace conditioning procedure in which the offset of a conditioned stimulus (CS) is followed 30 s later by an aversive foot shock unconditioned stimulus (US). Our results showed that cafeteria fed rats froze less than control rats when tested in the context where conditioning occurred ($P<0.01$); indicating that encoding of a hippocampally dependent trace was impaired in these rats. Conversely, cafeteria fed rats froze more than control rats when tested in a different context with the CS ($P<0.05$), demonstrating that when hippocampal function was compromised the cue was the best predictor of footshock. To examine underlying mechanisms, mRNA expression of inflammatory and neuroplasticity markers will be analysed within the hippocampus, as previous work has shown associations between hippocampal inflammation and behavioural deficits.

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POS-THU-096

CLAUSTRUM PROJECTIONS TO THE PREFRONTAL CORTEX IN THE CEBUS MONKEY (CEBUS APELLA)

Reser DH[1], Richardson K[1,2], de Oliveira Montibeller M[1], Zhao S[1]; Chan J[1], Soares JGM[3], Gattass R[3]; and Rosa MGP[1]

[1]Department of Physiology, Monash University, Clayton, VIC 3800, Australia
[2]Department of Psychology and Psychiatric Medicine, Monash University, Clayton, VIC 3800, Australia
[3]Programa de Neurobiologia, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro, Rio de Janeiro 21941-900, Brazil

The primate claustrum has a distinctive and peculiar geometry, which varies considerably across genera. We examined the pattern of claustrum projections following intracortical injections into orbital, frontal pole, and dorsolateral prefrontal cortex. Four adult cebus monkeys, a New World species, were injected with fluorescent retrograde neuroanatomical tracers. Brains were processed and sectioned using established techniques, and the pattern of labelled somae in the claustrum was assessed in relation to the complex 3-dimensional geometry of the primate claustrum. Prefrontal claustrum connections were extensive, and were largely concentrated in a discrete band in the rostral 2/3 of the claustrum, near the mid-level of its dorsal-ventral axis. Localization of major projections to dorsolateral and frontal cortex areas was consistent with previous descriptions of the marmoset, despite substantial differences in claustrum size and gross morphology between species. Evidence for segregation of claustrum neurons projecting to orbitofrontal and dorsolateral prefrontal areas was observed. We believe that elucidation of the claustrum-cortical connections across species will yield better understanding of functional networks between areas of primate cerebral cortex. [171 words]

POS-THU-097

PANAX GINSENG ATTENUATES OXIDATIVE–NITROSATIVE STRESS, INFLAMMATORY CASCADE AND COGNITIVE IMPAIRMENT IN RATS SUBJECTED TO MILD TRAUMATIC BRAIN INJURY: AN EVIDENCE OF NITRIC OXIDE SIGNALING

Puneet Rinwa[1], Anil Kumar[1]

1. Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh, INDIA

Oxidative stress and neuroinflammation, the prominent features of mild traumatic brain injury (mTBI), progressively evolves over time and are known to be the major contributors to cognitive deficits. The current study investigates the interaction of *Panax ginseng* (PG) against cognitive deficits and neuroinflammation associated with mTBI and the probable role of nitric oxide pathway in this effect. Wistar rats were subjected to weight drop injury and kept for a rehabilitation period of two weeks. Later, animals were administered with PG (50, 100, 200 mg/kg; p.o.) alone and in combination with NO modulators daily for another two weeks. mTBI caused significant memory impairment in Morris water maze task as evident from increased escape latency and total distance travelled to reach the hidden platform. This was followed by a significant decrease in frequency of appearance and time spent in target quadrant. Further, there was a significant rise in oxidative-nitrosative stress markers, inflammatory cytokines (TNF-α and IL-6), apoptotic factor (caspase-3) and acetylcholinesterase levels in both cortex and hippocampal regions of traumatized rat brain. PG (100, 200 mg/kg; p.o.) treatment significantly improved all these behavioral, biochemical, and cellular alterations. Further, pre-treatment of L-arginine (100 mg/kg; i.p.), a nitric oxide donor, with subeffective dose of AG (100 mg/kg; p.o.) reversed its protective effects. However, L-NAME (10 mg/kg, i.p.), a non-specific NO synthase inhibitor, potentiated the effects of PG. Our findings suggest that modulation of nitric oxide signalling cascade might be involved in the protective effects of PG against head trauma-induced cognitive impairment and neuroinflammation. (250 words)

POS-THU-098

MELANOCORTIN 3/4 RECEPTOR AGONIST REDUCES FOOD INTAKE BY ACTING ON VAGAL AFFERENT ENDINGS IN THE NUCLEUS OF THE SOLITARY TRACT.

Ritter R.C. and Campos C.A.

Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA 99164-7620

The melanocortin 3/4 receptor (MC4R) agonist, MTII, reduces food intake following injection into the fourth ventricle or nucleus of the solitary tract (NTS). Vagal afferent endings express MC4R, which enhance vagal afferent glutamate release. We postulated that that MTII-induced reduction of food intake is mediated MTII's action on vagal afferent endings in the NTS.

In intact rats fourth ventricle injection of MTII reduced food intake. A PKA inhibitor attenuated MTII-induced reduction of intake, suggesting that PKA-catalyzed phosphorylations might contribute to reduction of food intake. Consistent with this possibility, MTII increased synapsin 1 phosphorylation at serine 9, selectively in anterogradely labeled vagal afferent endings. This increase was prevented by PKA inhibition. MTII did not increase synapsin phosphorylation ipsilateral to unilateral nodose ganglion removal, suggesting that MTII acts directly on vagal afferents.

To determine whether vagal afferent endings are necessary for MTII-induced reduction of intake, rats received unilateral nodose removal, resulting in vagal afferent degeneration in the ipsilateral, but not contralateral, NTS. Unilateral nodosectomy attenuated reduction of food intake by MTII injected in the NTS ipsilateral to nodose removal, but not when MTII was injected into the contralateral NTS.

We conclude that reduction of food intake by hindbrain MC4R activation depends on vagal afferent endings. Moreover, close association between reduction of food intake by MTII and PKA-catalyzed synapsin 1 phosphorylation in vagal afferent endings suggests that increased synapsin phosphorylation could be a mechanism by which vagal afferent synaptic function is modulated by MC4R to control food intake. (248 words)

POS-THU-099

APOD GENE DELETION EXACERBATES THE PATHOLOGICAL PHENOTYPE OF APP/PS1 ALZHEIMER'S DISEASE MICE.

Hongyun Li[1, 2], K Ruberu[1, 2], T Karl[3, 4], B Garner[1, 2]

[1] Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW, Australia; [2] School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia; [3] Neuroscience Research Australia, Randwick, NSW, Australia; [4] School of Medical Sciences, University of New South Wales, NSW, Australia.

Apolipoprotein D (apoD) is an evolutionarily conserved lipocalin expressed in the brain. ApoD (and its homologues) protect against brain lipid peroxidation and extend lifespan in some organisms. Although increased expression of cerebral apoD is reported in Alzheimer's disease (AD), there is no evidence suggesting a protective role for apoD in an AD animal model. We crossed apoD knockout mice with APP/PS1 AD mice to generate APP/PS1 mice with wild type apoD levels "APP/PS1-WT" and littermates that lack apoD "APP/PS1-apoD$^{-/-}$". At 1 year of age, we compared AD-related pathological

alterations in these mice. We found a 53% increase ($p<0.05$) in amyloid (6E10 positive) plaque area in the hippocampus, and a significant increase of amyloid beta (Aβ) 40 (88% increase, $P<0.01$) and Aβ42 (3-fold increase, $P<0.01$) concentration in guanidine HCl-soluble fractions of hippocampal homogenates detected by ELISA in the APP/PS1-apoD$^{-/-}$ compared to APP/PS1-WT mice. Moreover,

a remarkable reduction of pre- and post-synaptic markers, synaptophysin (reduced by 45%, $P<0.05$) and post-synaptic protein 95 (reduced by 36%, $P<0.05$), was observed in the APP/PS1-apoD$^{-/-}$ mice. In conclusion, apoD deficiency exacerbates AD pathology in the APP/PS1 mouse and these data suggest a neuroprotective role for apoD in human AD. **(193 words)**

POS-THU-100

CAFFEINE MAINTAINS BASAL SYNAPTIC STRENGTH DURING 48H OF TOTAL SLEEP DEPRIVATION IN RAT HIPPOCAMPUS

Sahu S[1], Wadhwa M[1], Deep S[2], Thakur S[3], Nanda S[4], Panjwani U[1]

1. Neurophysiology Division, 2. Neurobiology Division, DIPAS, DRDO, Timarpur, Lucknow Road, Delhi, India, 3. Dept. of Biotechnology, Graphic Era University, Derhadun, India, 4. Ambedkar Center for Biomedical Research, Delhi University, Delhi, India. *E.mail: physiol.surajit@gmail.com*

Normal sleep-wake cycle ensures the balance of synaptic potentiation and depression which called total synaptic strength. Sleep deprivation significantly impairs the basal synaptic strength. A large body of evidence suggests that chronic caffeine treatment significantly improves synaptic plasticity during stress, including sleep deprivation. Here we investigated the impact of acute caffeine administration during 48h total sleep deprivation on basal synaptic strength in hippocampal region. Rats were sleep deprived using a novel sleep deprivation set-up by inducing cage shaking stimulus based on animal activity. After 48h the rats were subjected to one trial novel object recognition test or sacrificed to collect samples for western blotting, RT-PCR and immunohistochemical studies. SD induced a deficit in familiar object retrieval performance and caffeine administration during SD significantly improved object recognition memory. SD significantly decreased expression of BDNF, Gria2, Grin2a, c-JUN and PSD-95 in terms of both mRNA and protein level. Expression of total GluR1, CaMK-II and CREB mRNA/protein did not change after SD but phosphorylated GluR1-S831, CaMK-II, ERK1/2 and CREB significantly decreased in dorsal hippocampus. Caffeine administration during SD significantly improved phosphorylation of GluR1 at S831 position as well as level of P-CREB, P-CaMK-II and P-ERK 1/2 compared to SD. Caffeine administration during SD also prevented down regulation of BDNF, Gria2, Grin2a, MMP9, PSD-95, Synapsin 1, Synaptopodin and MAPK in mRNA or protein level or both. Overall, the results suggested that caffeine administration preserved basal synaptic strength and facilitated cognitive performance during 48h sleep deprivation. (241 words)

POS-THU-101

EARLY GESTATIONAL EXPOSURE TO MODERATE CONCENTRATIONS OF ETHANOL LEADS TO AN AGE-DEPENDENT DECLINE INATTENTIONAL PROCESSING IN C57BL/6J MICE

Sanchez-Vega M [1], Chong S[2], and Burne THJ[1]
[1]Queensland Brain Institute, University of Queensland, St Lucia, QLD. [2]Mater Medical Research Institute, QLD.

Alcohol consumption during pregnancy has deleterious effects on the developing foetus ranging from subtle physical deficits to severe behavioural abnormalities and is encompassed under a broad umbrella term, foetal alcohol spectrum disorders (FASD). High levels of exposure show distinct effects, whereas the consequences of moderate exposures have been less well studied. The aim of this study was to examine the effects of a moderate dose of ethanol exposure using an ad libitum drinking procedure during the first 8 days of gestation (GD0-8) in mice on attentional processing of offspring as they age.

Adult female C57BL/6J mice were mated and exposed to either 10% (v/v) ethanol or water for GD 0–8 and then offered water for the rest of gestation. Ethanol-exposed and Control mice underwent training for the 5 choice serial reaction time task in operant chambers at 3 months of age, and re-trained at 8 months of age.

There was no effect of Group on accuracy (% of correct responses) in 3-month-old mice. However, there was a significant effect of Group by Sex in 8-month-old mice, where EtOH-exposed males had significantly decreased accuracy ($p=0.005$), suggesting impaired attentional performance.

We are examining the molecular mechanism associated with this cognitive impairment by focussing on altered regulation of BDNF expression within the brain. We have previously shown a subtle phenotype in young mice using this model and these data suggest that there are longer term consequences of prenatal moderate exposure to ethanol with increasing age of the offspring. (250 words).

POS-THU-102

EFFECTS OF SHORT-TERM COGNITIVE ENRICHMENT ON COGNITION, ANXIETY- AND DEPRESSION-LIKE BEHAVIOUR

Singhal G[1], Jaehne EJ[1], Corrigan F[2], Baune BT[1]

1. Psychiatric Neuroscience Lab, Discipline of Psychiatry, School of Medicine, The University of Adelaide, Adelaide, Australia
2. Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, Adelaide, Australia

Objective: Exposure to cognitive enrichment (CE) has been shown to have distinct beneficial effects on the neurobiology and behaviour of animals. Such animals were observed to be more responsive and display diverse behaviour with improved cognitive performance. However, little is known about the effects of short-term CE on cognition, anxiety- and depression-like behaviour.

Methods: We have conducted experiments on C57BL/6 mice to measure the alterations in different behaviours in response to short-term CE with novel objects, toys and accessories. Three months old mice were reared in a cognitively stimulating enriched environment for four weeks followed by an assessment of a range of behaviours using an established battery of behavioural tests.

Results: In novel object recognition test, only enriched mice showed a slight preference for the novel compared to the familiar object, suggesting a higher retention and recognition memory. However, in other measures of learning and memory, the Y-maze and Barnes maze, both groups showed very similar behaviour. Results for anxiety- and depression-like behaviour also showed no differences between the two groups and both control and enriched mice showed similar locomotor activity throughout all tests in the behavioural battery.

Conclusion: The above results suggest that CE may play a role in memory; however no broad effects on various behaviours were observed at this young age. Therefore, further experiments on older mice and with longer duration of CE are required to fully elucidate the beneficial effects of CE on brain. (238 words)

POS-THU-103

THE CLAUSTRUM'S PROPOSED ROLE IN CONSCIOUSNESS IS SUPPORTED BY THE EFFECT AND TARGET LOCALIZATION OF *SALVIA DIVINORUM.*

Klaus M. Stiefel[1], Alistair Merrifield[2] and Alex Holcombe[3]

1. University of Western Sydney, The MARCS Institute, Sydney, NSW 2751, Australia.
2. NPS Medicinewise, Sydney NSW 2012, Australia.
3. University of Sydney, Department of Psychology, Sydney, Australia

We bring together three findings and ideas relevant for the understanding of human consciousness: (I) Crick's and Koch's theory that the claustrum is a "conductor of consciousness" center crucial for subjective conscious experience. (II) Subjective reports of the consciousness-altering effects the plant *Salvia divinorum*, whose primary active ingredient is salvinorin A, a κ-opioid receptor agonist. (III) The high density of κ-opioid receptors in the claustrum. Fact III suggests that the consciousness-altering effects of salvinorin A are due to a κ-opioid receptor mediated inhibition of primarily the claustrum and, additionally, the deep layers of the cortex, mainly in prefrontal areas. Consistent with Crick & Koch's theory that the claustrum plays a key role in consciousness, our analysis of the subjective effects of *Salvia divinorum* finds that salvia disrupts certain facets of consciousness much more than the largely-serotonergic hallucinogen LSD. Based on this data and on the relevant literature, we suggest that the claustrum does indeed serve as a conductor for certain aspects of higher-order integration of brain activity, while integration of auditory and visual signals relies on coordination by other areas including parietal cortex and the pulvinar.

POS-THU-104

PHARMACOLOGICAL INVESTIGATION OF THE ROLE OF mGlu5 AND NMDA RECEPTOR ON CONDITIONED FEAR.

Charlotte E. Handford[1,2], Shawn Tan[1,2], Andrew J. Lawrence[1,2], & Jee Hyun Kim[1,2]

1. Behavioural Neuroscience Division, Florey Institute of Neuroscience and Mental Health, VIC, Australia. 2. Florey Department of Neuroscience and Mental Health, Univerysity of Melbourne, VIC, Australia.

Metabotropic glutamate receptor 5 (mGlu5) and N-methyl-D-aspartate (NMDA) receptors play an important role in learning and memory through their role in underlying processes such as long term potentiation. Activation of mGlu5 leads to increased neuronal excitability and potentiation of NMDA currents in the structures important for fear conditioning such as the amygdala and hippocampus. This study examines the role of mGlu5 in acquisition and consolidation of conditioned fear to a tone and context in mice and further explores the relationship between mGlu5 and NMDA. Administration of the mGlu5 negative allosteric modulator 3-[(2-methyl-1,3,-thiazol-4-yl)ethynyl] pyridine (MTEP) pre-conditioning, but not post-conditioning, significantly attenuated cue-elicit freezing during fear conditioning. This effect was dose-related and not due to effects of MTEP on shock sensitivity. The NMDA receptor partial agonist D-cycloserine (DCS) was unable to recover this deficit, although post- conditioning injection of DCS alone facilitated the consolidation of fear. Overall these results indicate a crucial role of mGlu5 in acquisition and NMDA in consolidation of conditioned

POS-THU-105

INVESTIGATING THE POTENTIAL OF STEM CELL BASED THERAPY IN AN IMMUNOTOXIN MOUSE MODEL OF ALZHEIMER'S DISEASE

Tiwari D., Warden H., Haynes J.M., Nicolazzo J.A., Pouton C.W., Short J.L.
Monash Institute of Pharmaceutical Sciences, Parkville, Australia.

Purpose: To characterize a dual reporter embryonic stem (ES) cell line and validate an immunotoxin mouse model of Alzheimer's disease for future transplantation experiments. Methods: A dual (mcherry and $Lhx8^{+}$) reporter ES cell line was derived from E14Tg2a mouse ES cells, assessed for differentiation and characterized using immunocytochemistry. For the immunotoxin model, 6-8 weeks C57BL/6 male mice (n = 12) were treated with bilateral intracerebroventricular injections of saline or mu-p75-saporin toxin (0.4µg/µl/mouse) to cause cholinergic neuronal lesions. Mice were cognitively assessed using a novel water maze (WM) protocol and novel object recognition (NOR) paradigm. Immunohistochemistry was performed to detect toxin dependent neuronal loss. Results: A significant difference in learning WM task was observed during cued and spatial trials, with toxin-treated mice showing longer latency to platform than controls (two way ANOVA; $p<0.01$). Also performance during probe trial was significantly reduced in treated mice (t-test; $p<0.05$), indicating memory loss by toxin. No memory impairment was detected in NOR test. Immunohistochemistry for choline acetyltransferase (ChAT) confirmed a significant loss ($p<0.001$; t test) of cholinergic neurons in the medial septum. FACS analysis of reporter cell line showed a small population of $Lhx8^{+}$ cells studied at different time points of differentiation. Immunocytochemistry for ChAT on day 18 cells revealed few cholinergic positives neurons as compared to wild type controls. These data indicate that the model is appropriate for future transplantation studies. Conclusion: Literature suggests a possible role of Lhx8 in cholinergic development and these cells are being investigated through transplantation in our toxin model.

Word count: 250

POS-THU-106

LOCALIZING CATEGORY-SELECTIVE BOLD SIGNALS IN FMRI USING SWIFT

Koenig-Robert R[1], VanRullen R[2,3] and Tsuchiya N[1,4]

1. School of Psychology and Psychiatry, Monash University

2. Centre de recherche cerveau et cognition (CerCo), UMR5549, CNRS.

3. Université Paul Sabatier, CHU Purpan, Toulouse, France.

4. Decoding and Controlling Brain Information, Japan Science and Technology Agency, Chiyoda-ku, Tokyo, Japan

SWIFT (semantic wavelet-induced frequency-tagging) is a novel method, which periodically scrambles natural images in the wavelets domain, modulating the image's semantic content (object form) at a fixed temporal frequency (the tagging frequency) while conserving the image's principal low-level properties (such as luminance, contrast and spatial frequency). As such, SWIFT is hypothesized to be able to isolate high-level visual representations by directly modulating neural activity at the tagging-frequency without using subtractive approaches (which have been criticized due to their propensity to generate spurious results). While SWIFT has been successfully applied using EEG (Koenig-Robert & VanRullen 2013 Neuroimage), it is unclear whether SWIFT can specifically engage high-level category-selective regions without contribution from low-level visual areas.

To address the spatial specificity of SWIFT, we employed fMRI and localized brain areas selective for object categories (faces, houses and objects) in human participants (n=7) using slow tagging-frequencies (0.06, 0.08 and 0.1 Hz, counter-balanced across categories). We were able to frequency-tag the BOLD response using SWIFT and obtain category-specific responses. As we hypothesized, early visual areas were minimally engaged by SWIFT. On the other hand, a classic functional localizer (based on a subtractive approach) showed strong activations in V1/V2 in some cases. Category-specific activities elicited by SWIFT were mostly located beyond retinotopic areas and overlapped with canonical category-selective regions such as FFA and LOC, based on the classic localizer. We conclude that SWIFT is a valid method to localize category-selective regions in the human brain without using subtractive approaches. (245 words)

POS-THU-107

AMPHETAMINE AND SCOPOLAMINE IMPAIR PERFORMANCE OF DVD-DEFICIENT RATS ON A CONTINUOUS DETECTION TASK

Turner KM[1], McGrath JJ[1,2], and Burne THJ[1,2].

1. Queensland Brain Institute, The University of Queensland, St Lucia, Australia.
2. Queensland Centre for Mental Health Research, Wacol, Australia.

Developmental vitamin D (DVD) deficiency has been shown to affect attentional processing in adult rats. We have developed a novel continuous detection task (CDT) where omissions can be separated from inactivity and performance can be assessed using psychometric curves and signal detection theory indices. The aim of this study was to treat control and DVD-deficient rats with drugs known to disrupt attentional processing and assess performance on the CDT.
Adult male and female Sprague Dawley rats from control and vitamin D deficient dams (n=10/sex/group) were trained in operant chambers to perform the CDT. All rats were treated with amphetamine (0.75 and 1.25mg/kg, i.p.) and then scopolamine (0.05 and 0.1mg/kg, i.p.) with saline injections given between each dose.
DVD-deficient rats made more errors than controls ($F=6.69$, $p=0.015$) with increasing task difficulty (i.e. at short but not long stimulus durations) following 0.75mg/kg amphetamine. Overall performance was reduced to the same extent in both groups after 1.25mg/kg amphetamine ($F=2.39$, $p=0.047$). DVD-deficient rats had reduced accuracy compared to controls after 0.05mg/kg scopolamine at short stimulus durations ($F=4.56$, $p=0.040$). Performance at short stimulus durations was impaired across all groups after 0.1mg/kg scopolamine ($F=8.45$, $p=0.005$).
DVD-deficient rats were more sensitive than controls to the disruptive effects of amphetamine and scopolamine when attentional load was high. A dose-dependent reduction in accuracy and an increase in omissions was observed across all rats for both drugs. We could dissociate the disruptive effects of amphetamine and scopolamine using the CDT and selective attentional processing deficits were found in DVD-deficient rats. (250 words).

POS-THU-108

NEUROCOGNITIVE DISTURBANCES ASSOCIATED WITH ACUTE INFECTIOUS MONONUCLEOSIS, ROSS RIVER FEVER AND Q FEVER: A PRELIMINARY INVESTIGATION OF INFLAMMATORY AND GENETIC CORRELATES.

Cvejic E[1], Lemon J[1], Hickie IB[2], Lloyd AR[3], Vollmer-Conna U[1]

[1] School of Psychiatry, University of New South Wales, Australia

[2] The Brain and Mind Research Institute, School of Psychiatry, University of Sydney, Australia

[3] Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Australia

Neurocognitive disturbances are part of the acute sickness response to infection; however the underlying mechanisms remain unclear. We used a computerised battery to assess neurocognitive performance in subjects (N=107) enrolled in the Dubbo Infection Outcomes Study – a large prospective patient cohort followed from documented acute infection with Epstein Barr virus, Ross River virus, or *Coxiella burnetii* until complete recovery. Subjects were assessed when ill, and a subset again after complete recovery. Associations between sickness-related cognitive disturbances and single nucleotide polymorphisms in cytokine genes (interleukin [IL]-6, IL-10, tumor necrosis factor-α and interferon-γ) were explored. During acute infection, subjects exhibited slower matching-to-sample responses (p=0.03), poorer working memory (p=0.014), mental planning (p=0.045), dual task performance (p=0.02), and required longer to complete discordant Stroop trials (p=0.01) than after complete recovery. Objective impairments correlated significantly with self-reported symptoms as well as levels of the inflammation marker, C-reactive protein (p=0.001). Multiple regression analysis identified the high cytokine-producing G allele of the IL-6-174G/C polymorphism as an independent predictor of poorer neurocognitive performance when subjects were ill (p = 0.027). These findings confirm that acute infection impacts on neurocognitive performance manifesting as slowed responses and impaired performance on complex tasks requiring higher-order mental functioning; which has important real-world implications. The data additionally provide the first suggestive evidence for a role of a genetic predisposition to more intense inflammatory responses in objective neurocognitive disturbances during acute infections. (228 words)

POS-THU-109

THE EFFECT OF ADULT ENRICHMENT ON GOAL-ORIENTED PROBLEM SOLVING

Wakulicz A, O'Connor A M, Leamey C, Sawatari A

Physiology, Bosch Institute and School of Medical Sciences, University of Sydney, Sydney, NSW, 2006, Australia.

Animals exposed to an enriched environment during early life, experience an accelerated development of striatal neural circuitry and associated striatally-dependent behaviour. Anatomically, this acceleration is seen through the hastened development of perineuronal nets, markers of circuit maturity, which consolidate around inhibitory interneurons. Striatally mediated behaviours, such as goal-oriented learning and motor coordination, are also seen to develop precociously, and the positive effects of enrichment appear to persist into adulthood. Using the puzzle box test, a previously established method of measuring goal-oriented problem solving ability, we attempted to determine whether enrichment during adulthood could result in a similar improvement. Additionally, through immunohistochemical staining we compared the density of PNNs in the adult striatum between mice enriched during adulthood and mice raised in standard conditions. A mixed model ANOVA revealed that enrichment from birth had a significant effect on puzzle box performance ($F_{1,8}=10.595$, $p=0.012$), whereas enrichment during adulthood did not ($F_{1,7}=4.349$, $p=0.075$). Furthermore, enrichment during adulthood did not seem to have a significant effect on the density of PNNs in the adult striatum (2-tailed student's t-test, $p=0.4$). Our results suggest that enrichment during adulthood is not sufficient to cause significant improvements in puzzle box performance, nor does it result in a significant difference in the final density of PNNs in the adult striatum.

(211)

POS-THU-110

THE STRESS-REWARD INTERFACE: ROLE FOR RELAXIN-3/RXFP3 SIGNALLING IN STRESS-INDUCED ALCOHOL CONSUMPTION AND SUCROSE-SEEKING IN MICE

Walker AW[1,2], Chua BE[1], Smith CM[1,2], Gundlach AL[1,2], Lawrence AJ[1,2]

1 The Florey Institute of Neuroscience and Mental Health and 2 Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria, Australia

Objective: Relaxin-3/RXFP3 signalling can modulate alcohol-seeking in rats. While RXFP3-deficient (KO) mice exhibit normal baseline alcohol preference/consumption in a two-bottle choice paradigm, RXFP3 KO mice markedly reduce preference for/consumption of alcohol *c.f.* WT littermates following acute stressors. Here we extend these findings by examining the effect of RXFP3 deletion on sensitivity to alcohol and operant responding for sucrose and alcohol. Methods: In male WT and RXFP3 KO littermates (n=9/10), alcohol-induced motor impairment (ataxia) was assessed by filming mice walking on a treadmill after i.p. injection of either 2.0 g/kg alcohol or saline, and analysed using Digigait software. Sucrose and alcohol self-adminstration, motivational strength, and relapse-like behaviour were examined via operant conditioning experiments (n=10/treatment/group). Results: Ataxia: No differences in gait (such as hindlimb stance width) were observed between genotypes following saline or alcohol treatment ($p>0.05$). Alcohol did, however, significantly impact gait in WT and RXFP3 KO mice *c.f.* saline (i.e. hindlimb stance width; main effect of treatment, $p<0.001$), suggesting gait analysis technology can provide more detailed, reliable insights into alcohol-induced motor impairment in mice than other assays (e.g. rotarod). Operant conditioning: Compared to WTs, RXFP3 KO mice displayed fewer lever presses for sucrose reward during acquisition ($p=0.011$) and received fewer reward deliveries on a standard progressive ratio schedule ($p=0.046$), suggesting reduced motivation to obtain natural rewards. Analysis of alcohol self-administration data is ongoing. Conclusion: Global, whole-of-life RXFP3 deletion does not alter alcohol sensitivity, but RXFP3 signalling may promote reward-seeking in mice.

(240 words)

POS-THU-111

THE RELATIONSHIP BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE *NGFR* GENE AND SPORADIC ALZHEIMER'S DISEASE IN A CHINESE HAN POPULATION

Fan Zeng[1], Xiu-Qing Yao[1], Xu Yi[1], Hong-yuan Cao[1], Xin Wang[1], Shu-Sheng Jiao[1], Qing-Hua Wang[1], Hua-Dong Zhou[1], Xin-Fu Zhou[2], Yan-Jiang Wang[1]

1. Department of Neurology and Center for Clinical Neuroscience, Daping Hospital and Institute of Field Surgery, Third Military Medical University, Chongqing 400042, China. 2. School of Pharmacy and Medical Sciences, Faculty of Health Sciences, University of South Australia, Adelaide, SA, Australia.

Increasing evidence shows that p75 neurotrophin receptor (p75NTR, encoded by *NGFR* gene) plays diverse roles in the pathogenesis of Alzhermer's disease (AD), such as the production of amyloid-beta, neuronal death, neurite degeneration, tau hyperphosphorylation and cognition decline. To assess the association between single nucleotide polymorphisms (SNPs) of the *NGFR* gene and sporadic AD (sAD) in the Chinese Han population, a case-control study was conducted including 220 sAD patients and 245 controls. 12 tag SNPs covering the entire *NGFR* gene were selected through construction of linkage disequilibrium blocks, and genotyped with a multiplex polymerase chain reaction-ligase detection reaction (PCR-LDR) method. We found that rs2072446 was significantly associated with an increased risk of sAD (additive model: OR=1.99, 95%CI=1.27-3.14) adjusted by age, sex and APOE status. And this association remained significant after correction for multiple tests. This result suggested that inherited polymorphisms within the *NGFR* gene were associated with the risk of AD. According to the position and predicted function of rs2072446, it may affect the risk of AD through inhibiting the shedding of p75NTR extracellular domain. (174 words).

POS-THU-112

HIGH FREQUENCY (>25 Hz) PEAKS IN SCALP ELECTRICAL RECORDINGS.

Willoughby JO[1], Whitham EM[1,], Fitzgibbon SP[1,2], Grummett TS[2], Dylan DeLosAngeles[2], Lewis TW[2], Pope KJ[2].

1. Centre for Neuroscience and School of Medicine, Flinders University and Medical Centre, GPO Box 2100 Adelaide, South Australia 5001, Australia. 2. School of Computer Science, Engineering and Mathematics and Medical Device Research Institute, Flinders University, GPO Box 2100 Adelaide, South Australia 5001, Australia

Published power spectra of electroencephalographic (EEG) activity recorded from the scalp rarely demonstrate peaks of power in the gamma range (25-100 Hz). Here we describe EEG spectral peaks observed in a systematic study of EEG spectra from controls and from subjects with neuro-psychiatric diseases.

High sample-rate, 128-channel, scalp electrical activity was recorded from 500 subjects with neuropsychiatric diseases and from 100 subjects without a CNS disorder. Recordings were made during cognitive tasks, including eyes-open and eyes-closed states, and converted to power spectra. Peaks of power were determined visually and algorithmically. In some subjects with identified peaks, 24-hour recordings were made to determine if they were persistent.

In controls and in patients with neuro-psychiatric disease, 22% exhibited peaks of power at or above 25 Hz. Peaks were most prominent in central and frontal regions. They were present during wakefulness and rapid eye-movement sleep, but were decreased in power and/or frequency during slow-wave-sleep. The frequency distribution of peaks showed separation between alpha and higher frequency, but no separation between beta and gamma.

The results show that gamma EEG can be expressed with concentrations of power in narrow or wide frequency bands and not have a frequency distribution range that can be separated from beta. These findings extend the described range of rhythmic EEG components. (212 words)

POS-THU-113

AUDITORY STIMULATION MODULATES AMYGDALA NETWORK DYNAMICS

Windels F, Stratton P, Sah P.
Queensland Brain Institute, The University of Queensland, Brisbane, Queensland.

Auditory fear conditioning is a paradigm that is widely used to investigate the physiology of associative memory. Based on this model the co-activation of aversive and sensory inputs converging onto neurons of the lateral amygdala is proposed to induce synaptic plasticity that supports fear learning. However, the underlying amygdala network dynamics remain unexplored. In this study, male Sprague-Dawley rats (n=4) were implanted with 8 tetrodes targeting the lateral amygdala. A screening period started a week after surgery and consisted of repeated presentation of tones at different frequencies (3 to 12 KHz). Z-scored peristimulus-time-histograms were used to analyse the responses of single units to the tones (bins: 20ms; 500ms baseline; $p<0.01$). We found that half of the tone-responsive cells (n=50) responded to only one frequency whereas the remaining cells responded to up to 8 frequencies. Despite the use of a broad range of frequencies, overlapping the peak sensitivity of the rat audiogram, we did not find a preferential response to one frequency based on the number of responsive cells or the response amplitude. When compared between sessions we found that only 30% of frequency responses (per unit) were conserved. Furthermore, cross-correlation analysis of small network (number of units > 5) showed that coupling between neurons increased during tone presentation independent of frequency (one-way ANOVA, $p<0.01$). Taken together, these results indicate that auditory stimulation induces network dynamics in the amygdala that are synergistically combining single-unit tone responses.

(235 words)

POS-THU-115

ARIPIPRAZOLE REDUCES CUE-INDUCED REINSTATEMENT OF COCAINE SEEKING BY ENHANCING CUE EXTINCTION LEARNING IN ADOLESCENT RATS

Zbukvic I, Perry C, Lawrence AJ, Kim JH

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

Much lifelong substance abuse begins with drug use during adolescence. Critically, once addicted, adolescents are more resistant to treatment interventions and more liable to relapse. Relapse is commonly triggered by drug-associated cues, the salience of which is mediated in part by dopamine 1 receptor (D1R) signalling in the prefrontal cortex (PFC). The adolescent PFC is dominated by D1R compared to 2 (D2R) signalling and is therefore naturally primed to attribute stronger salience to drug-associated cues. Aripiprazole, an anti-psychotic drug commercially branded Abilify^{TM}, exhibits high affinity partial agonism for D2R and may be used to restore D1/D2R balance in the adolescent brain. We examined the acquisition, extinction and reinstatement of cocaine-seeking behaviour in adolescent rats using an intravenous-self administration paradigm. Postnatal day (P)34 (±1) rats were trained to lever press for cocaine (3mg/kg/infusion) over 10-14 days, where cocaine infusions were paired with a light cue. Once stable self-administration was established, rats received 7 daily lever extinction sessions, where lever pressing had no programmed consequences. Next day, rats received a systemic injection of either 5mg/kg Aripiprazole or saline before undergoing a single cue extinction session consisting of 120 cue-alone presentations with levers retracted. Next day, all rats were tested for cue-induced reinstatement, in the absence of cocaine. Results showed Aripiprazole injected prior to cue extinction significantly reduced cue-induced reinstatement compared to saline ($ps < .05$), suggesting that acutely redressing adolescent D1/D2R imbalance can improve cue extinction learning. Aripiprazole therefore represents a novel therapeutic strategy to enhance cognitive treatments for adolescent drug users. (250 words)

POS-THU-116

MANIPULATION OF AXON GUIDANCE CUE, EPHRINA5, AFFECTS THE INTEGRATION OF DOPAMINERGIC NEURAL GRAFTS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

Kauhausen JA[1], Rodger J[2] Thompson L[1], Parish CL[1]

[1] Stem Cells and Neural Development Laboratory, Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Australia. [2] School of Animal Biology, The University of Western Australia, Australia.

Both attractive and repellent cues are required to assist axons to grow to their correct target within the brain. These cues are not only required for development, but are likely to be required for regeneration. Following the completion of development, the expression of many guidance cues is significantly decreased. Interestingly it has been observed that many CNS neurons will continue to express the receptors for guidance cues into adulthood, implying that these same neurons may remain responsive to these cues throughout life and that there are additional roles for guidance cues beyond that of initial development.

EphrinA5 is a ligand known to play a role in regulating DA axon growth and guidance during development. Our work aimed to determine whether manipulation of EphrinA5 could influence the outgrowth of a fetal dopaminergic graft in a mouse model of Parkinson's disease, as insufficient reinnervation of the host brain has been a major limitation in cell based therapy for PD. In loss- and gain-of-function studies, we have found that EphrinA5 plays a significant role in graft integration. We demonstrate that loss of EphrinA5 significantly decreases the area of the striatum innervated by the graft and the density of the dopaminergic fibers whilst having no effect on cell survival. Conversely, EphrinA5 over-expression in the host tissue promoted regeneration. These findings hold significant implications for enhancing reinnervation following neural injury.

POS-THU-117

THE FIBROTIC SCAR IS AN IMPEDIMENT FOR AXONAL REGENERATION AFTER TRAUMATIC BRAIN INJURY.

Kawano H[1], Kimura-Kuroda J[1], Komuta Y[1], Li H-P[2], Takeuchi K[3]

[1]Department of Brain Development and Neural Regeneration, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. [2]Department of Human Anatomy, College of Basic Medical Sciences, China Medical University, Shenyang, China. [3]Department of Molecular Biology and Biochemistry, School of Medicine, Niigata University, Niigata, Japan.

In the central nervous system (CNS) of adult mammals, transected axons display almost no regenerative capacity following traumatic injury. Various kinds of factors which occur around the lesion site, such as glial scar and chondroitin sulfate (CS) proteoglycans, have been postulated to prevent the regrowth of severed axons. We have demonstrated that suppression of formation of the fibrotic scar which contains deposition of type IV collagen (Col IV) is required for axonal regeneration in the damaged CNS in a variety of animal models. These include (1) suppression of Col IV synthesis, (2) newborn mouse, (3) the mouse hypothalamic arcuate nucleus, (4) enzymatic degradation of CS, (5) transplantation of olfactory ensheathing cells, and (6) suppression of transforming growth factor-β (TGF-β) function (reviewed in Kawano et al., Cell Tissue Res., 349:169-180, 2012).

Addition of TGF-β1 to the coculture of meningeal fibroblasts and cerebral astrocytes induced a fibrotic scar-like cell cluster which repels neurites of cerebellar neurons (Kimura-Kuroda et al., Mol. Cell. Neurosci. 43: 177-187, 2010). The fibrotic scar and TGF-β1-induced cell cluster intensely expressed both CS and dermatan sulfate (DS). Administration of enzymes specifically degrading DS or CS in injured brains and in cell culture demonstrated that DS is involved in the fibrotic scar formation and CS inhibits axonal regeneration (Li, Komuta et al., J. Neurotrauma, 30: 413-425, 2013). We propose that the suppression of fibrotic scar formation would be a reliable strategy to promote axonal regeneration in the damaged CNS.(239 wordds).

POS-THU-118

COMPLEX ROLES FOR TEN-M3 IN THE GENERATION OF THALAMOSTRIATAL CIRCUITRY

Tran H, Sawatari A and Leamey CA
Physiology, School of Medical Sciences and Bosch Institute, University of Sydney, NSW, 2006

Ten-m3 is expressed in the parafascicular thalamic nucleus (PFN), and in patches in its major target, the striatal matrix. Further, thalamostriatal axons do not show their characteristic clustered pattern within the matrix of Ten-m3 knockouts (KOs). We report here that Ten-m3 positive patches in the matrix overlap substantially with clusters of thalamostriatal axons in wildtype (WT) mice, suggesting a causal relationship. We also show that the patchy pattern in the matrix is overlayed on a high-dorsal to low-ventral gradient, which corresponds topographically with a similar gradient in the PFN. The timecourse of expression is also similar in both structures, commencing by embryonic day (E)17 and becoming more robust by postnatal day (P)3. Tracing studies show that in WT mice, thalamic axons first extend terminal branches into the striatum at E17. Ingrowth is delayed in KOs, with a significant decrease in the number of branches formed at E17 (WT: 1478.8 ± 346.87, n = 3; KO: 684.4 ± 325.1, n = 3, p = 0.04, t-test). By P3, thalamostriatal terminals occupied more of the striatum, suggesting a decrease in specificity of the projection by this stage. We also investigated the distribution of a major target of thalamostriatal axons, cholinergic interneurons. We find a significant decrease of these cells specifically in ventrolateral striatum (WT: 88.83 ± 4.78, n = 3; KO: 62 ± 4.19, n = 4; $F(1,4) = 14.22$, p = 0.013, repeated measures ANOVA). Our data point towards multiple, complex roles for Ten-m3 in the generation of thalamostriatal circuits. (249 words)

POS-THU-119

THE AXON GUIDANCE RECEPTOR NEOGENIN REGULATES CADHERIN ENDOCYTOSIS AND ADHEREN JUNCTION ASSEMBLY

Lee NK, Nourse C, Cox H, Cooper HM

Queensland Brain Institute, University of Queensland, Brisbane, Australia

The cadherin cell adhesion receptors are essential in establishing adherens junctions (AJ) between radial glial progenitors that derive from the neuroepithelium in the embryonic brain and give rise to all neurons in the CNS. Failure in AJ formation due to loss of cadherin destroys progenitor morphology and results in the failure to generate neurons. Our previous studies revealed that depletion of the axon guidance receptor Neogenin (Neo) in the embryonic mouse cortex results in severe disruption of progenitor morphology due to a failure in AJ assembly, leading to the formation of heterotopias protruding into the ventricle and below the cortical plate. To determine the role of Neo in cadherin adhesion, we used small-interfering (siRNAs) to knockdown Neo in CaCo2 cells, a well-established *in vitro* epithelial cell model for the study of AJ formation. We found that loss of Neo prevents E-cadherin homophilic adhesion between adjacent cells and results in the formation of large intracellular E-cadherin-positive vesicles adjacent to the failed junctions. Vesicular trafficking whereby cadherin undergoes endocytosis and recycling to the plasma membrane via recycling endosomes regulates cell surface cadherin levels at the AJ. Using a surface biotinylation assay, we show that Neo knockdown in CaCo2 cells results in a decreased rate of E-cadherin endocytosis over 30 minutes compared to CaCo2 cells transfected with scrambled siRNA (endocytic rate constant: control 0.2units/sec, knockdown 1.0units/sec, $p<0.003$, $n=5$). These results suggest Neo plays a critical role in cadherin trafficking and AJ assembly between radial progenitors within the neural stem cell niche. (248 words).

POS-THU-120

MODULATING THE IMMUNE RESPONSE TO IMPROVE FUNCTIONAL RECOVERY, TISSUE SPARING AND DONOR CELL SURVIVAL FOLLOWING SPINAL CORD INJURY

Lovett SJ[1], Harvey AR[1], Plant GW[2], Hodgetts SI[1]

1. Spinal Cord Repair Laboratory, University of Western Australia, 2. Stanford Partnership for Spinal Cord Injury and Repair, Stanford Institute for Neuro-Innovation and Translational Neurosciences and Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

Spinal cord injury (SCI) is biphasic. The primary injury involves the initial mechanical insult to the spinal cord resulting in bruising of the cord, damage and/or demyelination of axons, cell death and haemorrhaging at the injury site. Secondary injury events contribute to the spread of damage along the spinal cord and are initiated by the inflammatory immune response of the body. Immediately following injury, pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukins 1β and 6 (IL-1β, IL-6) are released from damaged cells and resident cells of the spinal cord, driving the inflammatory response.

We have previously shown a marked improvement in functional (locomotory) and morphological outcomes in host tissue following transplantation of Stro-1^{+} adult human mesenchymal precursor cells (hMPCs) into the contused spinal cord in Nude rats. However, donor hMPCs do not survive beyond 1 month due to the host immune response. Modulating pro-inflammatory cytokine levels and immune cell activity immediately following injury may lead to decreases in secondary degeneration and increased donor hMPC cell survival. Here we investigate the effects of Enbrel, a TNFα antagonist, administered after a moderate contusive SCI. We present preliminary data that shows moderate improvement in functional outcomes (BBB score) from 5 weeks post injury with Enbrel treatment, and are currently performing combinatorial treatments with hMPC transplantation that shows functional improvement at 3-4 weeks post-transplantation ($p<0.05$). Targeted antibody depletion of specific immune cells (to further enhance hMPC survival) alone and in combination with Enbrel treatment is also underway. (246 words).

POS-THU-121

QUANTITATIVE FLOW CYTOMETRIC ANALYSIS OF RAT EMBRYONIC DOPAMINE PRECURSORS AND MATURE NEURONS

Luan W[1,2], Alexander SA[1,2], Eyles DW[1,2],
1. Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia
2. Queensland Centre for Mental Health Research, Brisbane, QLD, Australia

Our group have suggested that the developmental absence of vitamin D may affect how dopamine (DA) neurons develop as a putative convergent mechanism in schizophrenia. Our maternal vitamin D deficiency model has revealed reduced transcriptional level of Nurr1, an essential factor to induce tyrosine hydroxylase (TH) expression and for DA neuron differentiation, in mesencephalon (MS) at early developmental ages. To establish a quantitative flow cytometric (FCM) method to assess the relative proportion of DA cells in rat MS at specific developmental ages. Ventral and dorsal regions of MS were dissected respectively from embryonic day 15 (E15) foetuses from timed pregnant Sprague-Dawley rats. For FCM analysis, MS cells were prepared by papain enzyme and mechanical dissociation. Cells were fixed and permeablized. We applied anti-TH specific antibody combining Alexa-488 or Alexa-647 labeled secondary antibody for fluorescent labeling. Stained MS cells were analyzed by BD LSR II flow cytometer and sorted by BD Influx Cell Sorter. Images of sorted cells were taken by Axio Imager Azure microscopy. We are able to distinguish 3.5-5% TH positive cells from ventral MS based using flow cytometric immunophenotyping analysis. This compared to less than 1% from the more dopaminergically-poor dorsal MS. Specific fluorescent signal in TH positive cells sorted by BD Influx Cell Sorter was confirmed under microscopy. This quantitative FCM phenotyping provides an easy and quick approach assessing DA neuronal cell number from rat embryonic MS using protein marker specific antibody. Additionally it allows the simultaneous quantitation of DA progenitors, precursors and mature neurons.

POS-THU-122

DEFINING THE REGULATION OF AMYLOID PRECURSOR PROTEIN N-TERMINAL FRAGMENT GENERATION

Luu, Luan, Ciccotosto, G.D. and Cappai, R.

Department of Pathology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia

The Amyloid Precursor Protein (APP) is a type 1 transmembrane protein that undergoes proteolytic cleavage including the generation of the amyloid β peptide which is central to Alzheimer's disease (AD). Since the function of the other APP metabolic products is less well known, it will be important to decipher their physiological roles in order to properly understand the diverse roles of APP. We have described the processing of APP at its amino terminal region to generate secreted N-terminal fragments (NTFs) with a relative molecular weight of 17-28 kDa. It is believed the metalloprotease, meprin-β is responsible for cleaving APP to produce the NTFs. While the precise amino acid sequence is unknown, based on its size and antibody reactivity profile, the NTFs would contain the N-terminal growth factor domain suggesting it would possess neurotrophic functions. Our aims included defining the function of APP by determining the regulation of APP processing into NTFs and to define their neurological function. We will show that when SH-SY5Y cells have increased expression of APP dimerization, NTF levels in the media decreased causing changes in cell morphology including a major decrease in neurite outgrowth. Conversely, by inhibiting APP dimerization, this caused and an increase in secreted NTF levels which promoted neurite outgrowth. Moreover, meprin-β overexpression in SH-SY5Y cells resulted in increased secreted NTF levels and concomitant increased neurite outgrowth. We propose that the NTF's are important for neurtiogenesis and neuroprotection.

POS-THU-124

TIMING OF SUB-CHRONIC ANTIPSYCHOTIC EXPOSURE DIFFERENTIALLY AFFECTS BRAIN FUNCTION

Moe AAK[1], Kurniawan ND[2], Alexander S[1], Burne THJ[1,3], Eyles DW[1,3].

1. Queensland Brain Institute, The University of Queensland, Australia. 2. Centre of Advanced Imaging, The University of Queensland, Australia. 3. Queensland Centre for Mental Health Research

Antipsychotic drugs (APDs) are increasingly being used in children and adolescents for a variety of psychiatric conditions, such as autistic spectrum disorder and schizophrenia. However, there is a paucity of studies directly comparing the effect of age of exposure to APDs on adolescent brain development. We have used a preclinical longitudinal study in rats to examine the consequences of subchronic exposure (daily injections for 21 days) to three commonly used APDs (haloperidol, HAL; clozapine, CLZ; risperidone, RIS) either as adolescents, young adults or adults. We assessed behavior using a conditioned avoidance response (CAR), which is a well-validated behaviour impaired by APDs. When tested on day 17 of APD treatment, CAR was significantly disrupted by 0.6mg/kg/day RIS in adolescents ($p<0.05$) and adults ($P<0.01$), and 0.05mg/kg/day HAL in all three ages ($p<0.01$). By contrast, 15mg/kg/day CLZ induced a tolerance-like condition without any profound CAR impairment in any age group. After a lengthy drug washout period (>14 days), CAR was again examined to assess long-term effects of APDs on brain function. Challenge with half dose of the corresponding drug induced significant CAR impairment only in RIS-treated adolescents but not with other drugs or age groups. Our results indicate that both the age of exposure and the drug type determine the nature of acute and long-term outcomes on integrated brain function. These behavioural data will be further correlated with the data on brain structural changes from longitudinal live magnetic resonance imaging and dopamine neurotransmission dynamics. (241 words).

POS-THU-125

ANALYSING THE BIOLOGICAL FUNCTION OF PS2V: AN ABERRANT SPLICING PHENOMENON OR EVOLUTIONARILY CONSERVED MECHANISM ?

[1]Hani Moussavi Nik[1], Morgan Newman, [1]Lachlan Wilson, [2]Esmaeil Ebrahimie [3,4,5]Giuseppe Verdile, [6]Gavin Chapman, [3,4,5]Ralph Martins and [1]Michael Lardelli.

[1]The School of Molecular and Biomedical Science, The University of Adelaide. [2]Technology Enhanced Learning Group, University of South Australia. [3]Centre of Excellence for Alzheimer's Disease Research and Care, School of Exercise, Biomedical and Health Sciences, Edith Cowan University. [4]Sir James McCusker Alzheimer's Disease Research Unit, Hollywood Private Hospital. [5]School of Psychiatry and Clinical Neurosciences, University of Western Australia. [6]Developmental Biology Division, Victor Chang Cardiac Research Institute

Alzheimer's disease (AD) can be classed as familial (FAD, usually with early onset) or sporadic (mostly late onset). Most FAD mutations occur in the *PRESENILIN* genes. These genes are required for Amyloidβ peptide (Aβ) production through their role in γ-secretase cleavage of AMYLOID PRECURSOR PROTEIN. A variety of evidence implicates hypoxia as a contributing factor to sporadic AD. We have shown that the mechanism regulating PS2V induction is conserved during evolution. We find that zebrafish possess a PS2V-like isoform, PS1IV, produced from the fish's *PSEN1* orthologous gene rather than its *PSEN2* orthologue. We show that the molecular mechanism controlling formation of PS2V/PS1IV is conserved since the divergence of teleosts and tetrapods and was probably present in the common ancestor of the *PSEN1* and *PSEN2* genes. The structures of human PS2V and zebrafish PS1V have diverged greatly but their ability to stimulate γ-secretase activity and suppress the unfolded protein response is conserved. We also show that microarray analysis of PS1IV function reveals modulation of inflammatory and vascular responses and other functions. (270 words)

POS-THU-126

EPH/EPHRIN SIGNALLING INFLUENCES THE COMPLEX SPATIOTEMPORAL DEVELOPMENT OF THE NONHUMAN PRIMATE VISUAL CORTEX

Mundinano IC, Kwan W, Bourne JA

Australian Regenerative Medicine Institute, Monash University

The traditional view of visual cortex development proposes a hierarchical sequence in which the primary area develops first driving the subsequent development of higher–order areas. However, recent evidence from the nonhuman primate suggests that particular extrastriate areas develop in parallel to the primary visual cortex. The precise molecular events driving this precocious development have yet to be determined. We suggest that Eph/Ephrin guidance signaling plays an integral role. Employing the complex visual system of the marmoset monkey (*Callithrix jacchus*), we anatomically profiled the postnatal spatiotemporal development of the visual cortex and thalamic nuclei utilizing immuno-histochemistry. Cerebral tissues from marmosets aged PD0, PD14, PD28, PD42, PD93 and adult were first reacted for antibodies against the neuronal subtype markers calbindin (Cb), parvalbumin (Pv) and nonphosphorylated neurofilament (NNF). These proteins have previously been demonstrated to be associated with particular stages in cortical development. Subsequently, expression profiles for the receptor EphA4 and ligands Ephrin A5, Ephrin A2 and Ephrin B1 were obtained and examined in combination with the results for the neuronal subtype markers. During postnatal maturation, the dorsal ‘stream’ associated areas - the dorsomedial and middle temporal visual cortical areas, revealed a higher expression of Cb, Pv and NNF than ventral stream visual cortices. This corresponded with a discrete expression of the ephrins in these areas that subsequently decreased in level of expression or became homogenous by adulthood. These results indicates that postnatal maturation of visual dorsal ‘stream’ precedes the ventral ‘stream’ areas, and it is strongly influenced by the Eph/Ephrin signalling.

POS-THU-127

OLIGODENDROGLIAL DELETION OF TrkB AT DIFFERENT STAGES OF DEVELOPMENT EXERTS DISTINCT INFLUENCES UPON CNS MYELINATION

Ferner AH, Wong AW, Xiao J, Peckham H, Giuffrida L, Wood R, Kilpatrick TJ, Murray SS.
Department of Anatomy and Neuroscience, and The Florey Institute of Neuroscience, The University of Melbourne.

We have previously identified that the neurotrophin brain-derived neurotrophic factor (BDNF) promotes oligodendrocyte myelination *in vitro* via activating oligodendroglial-expressed tropomyosin related kinase B (TrkB) receptors. The objective of this study was to assess whether this mechanism also drives myelination *in vivo*. To address this we have generated mice subjected to oligodendroglial-specific deletion of the TrkB receptor at different stages of development and found this exerts distinct effects upon myelination. Deletion of TrkB in mature oligodendrocytes, under the influence of the MBP promoter ($TrkB^{fl/fl}$ MBP-cre) results in a significant hypomyelinating phenotype during early postnatal development which normalises by approximately one month of age. In contrast, deletion of TrkB in oligodendrocyte precursor cells, under the influence of the CNPase promoter ($TrkB^{fl/fl}$ CNPase-cre) results in normal myelin development. These data demonstrate that deletion of TrkB at different stages in the oligodendroglial lineage exerts distinct influences upon myelination. We have generated a third line, in which TrkB is deleted in oligodendroglial progenitor cells, under the influence of the Olig2 promoter ($TrkB^{fl/fl}$ Olig2-cre), and are currently assessing myelin development in these mice. Our data indicate that TrkB exerts an important influence to promote myelination by mature oligodendrocytes *in vivo*, and suggests that there are compensatory mechanisms at play when TrkB is deleted earlier in the oligodendroglial lineage. (212 words).

POS-THU-128

NFIX REGULATES BOTH PROLIFERATION AND MIGRATION WITHIN THE SUBVENTRICULAR ZONE OF THE POSTNATAL BRAIN

Heng Y. [1], Gronostajski, R.M. [3], Richards L.J. [1,2] and Piper M. [1, 2]

[1]The School of Biomedical Sciences, [2]Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

[3]Department of Biochemistry and the Program in Neuroscience, Developmental Genomics Group, New York State Center of Excellence in Bioinformatics and Life Sciences, State University of New York at Buffalo, New York, USA.

Neurogenesis within the adult brain occurs in two primary areas, the subventricular zone (SVZ) lining the walls of the lateral ventricles, and the subgranular zone of the hippocampal dentate gyrus. Within the SVZ, neural progenitor cells give rise to neuroblasts that migrate through the rostral migratory stream (RMS) into the olfactory bulb (OB), where they differentiate into interneurons. However, our understanding of the molecular regulation of these fundamental events remains limited. Here we demonstrate that the transcription factor Nuclear factor one X (NFIX) plays a key role in multiple aspects of SVZ development in the postnatal brain. Mice lacking *Nfix* display a dramatically enlarged SVZ at postnatal day 20. Moreover, expression of the Notch effector genes, *Hes1* and *Hes5,* are markedly increased within the SVZ of the mutant mice, indicative of increased proliferation within this zone in the absence of *Nfix*. Interestingly, we also reveal that the OB of *Nfix*$^{-/-}$ mice is significantly smaller than controls, suggestive of impaired migration of SVZ-derived neuroblasts along the RMS. We reveal that both cell-autonomous and non-cell-autonomous factors contribute to this phenotype, indicating that *Nfix* contributes in a variety of different ways to the development of this neurogenic niche. Finally, we reveal that *Gdnf,* a factor expressed within the RMS that promotes neuroblast migration, is a target for transcriptional activation by NFIX, providing a mechanism through which NFIX mediates one facet of SVZ development. Collectively, these data show that NFIX regulates multiple aspects of SVZ development, highlighting the pivotal role this transcription factor plays in the development of this neurogenic niche of the postnatal brain.

POS-THU-129

EFFECTS OF ENRICHMENT ON PNN FORMATION AND PV+ EXPRESSION IN DORSAL AND VENTRAL HIPPOCAMPUS

Rahman T, O'Connor A, Leamey CA, Sawatari A
Discipline of Physiology, School of Medical Sciences and Bosch Institute, University of Sydney, Australia

Environmental enrichment (EE) has been shown to improve sensory, motor, and cognitive performance, as well as accelerate the timing of 'critical periods'. The closure of these developmental epochs has been associated with the formation of perineuronal nets (PNNs) around parvalbumin positive (PV+) inhibitory interneurons. Our previous findings revealed that the appearance of PNNs within the dorsal hippocampus was expedited when mice were enriched from birth. How EE affects the formation of these markers within other subregions of the hippocampus, however, has yet to be determined. Accordingly, brains from P10, P15, P21 and adult C57BL6J pups raised in either enriched (EE) or standard environments (SE) from birth, were sectioned (coronally and horizontally) processed for WFA (a lectin that binds to PNNs) and PV+ antibody labeling within the hippocampus and compared across region and enrichment cohort. Analysis of adult sections has revealed that PNN number as well as PNN/PV+ overlap are significantly greater in dorsal compared to ventral hippocampus (PNNs: $F(1, 9) = 12.836$, $p = 0.006$; Overlap: $F(1, 9) = 15.827$, $p = 0.003$). PV+ cells, on the other hand, are significantly greater in EE compared to SE mice ($F(1, 9) = 39.184$, $p < 0.0001$). Our preliminary findings suggest that EE affects PV+ density within the hippocampus, and may affect the appearance of PNNs in differing ways. (217 words)

POS-THU-130

HOW DOES EARLY POSTNATAL ACTIVITY INFLUENCE CONTRALATERAL TARGETING OF CALLOSAL AXONS?

Rodrigo Suárez[1†], Laura R. Fenlon[1†], Roger Marek[1], Lilach Avitan[1], Pankaj Sah[1,2], Geoffrey J. Goodhill[1,3] & Linda J. Richards[1,2].
[1]Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia. [2]School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia. [3]School of Mathematics and Physics, The University of Queensland, Brisbane, QLD 4072, Australia. [†] These authors contributed equally to this work

Bilateral integration of sensory and associative brain processing is achieved by the formation of precise connections between hemispheres via the corpus callosum. These connections form postnatally, and unilateral ablation of either sensory-evoked or spontaneous cortical activity during a critical period severely affects the pattern of callosal wiring. However, little is known about how this early activity affects formation of precise circuits. Here, we quantified contralateral callosal targeting under manipulations altering sensory (n=14) or cortically driven (n=16) activity during a critical period of postnatal development. We found that rather than absolute levels of activity, callosal axon targeting requires similar patterns of activity in the two hemispheres ($p<0.05$). We further confirmed the functional disruption and rescue of callosal projections between treatments with optogenetic mapping of interhemispheric circuits (n=18). Moreover, we found that the spatial arrangement of intact barrels (n=21) directs the formation of region-specific callosal projections ($p<0.01$). Our findings reveal a novel principle governing commissural wiring, in which early patterned activity between hemispheres directs callosal axon targeting. (165 words)

POS-THU-131

BILATERAL CONNECTIONS IN THE MARSUPIAL BRAIN ARE SPATIALLY SEGREGATED ACCORDING TO THEIR PLACE OF ORIGIN

Rodrigo Suárez[1], Laura Morcom[1], Nyoman Kurniawan[2] & Linda J. Richards[1,3]
[1]Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia. [2]Centre for Advanced Imaging, The University of Queensland, Brisbane, QLD 4072, Australia, [3]School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.

Precise integration of left and right brain hemispheres is crucial for sensory, motor and associative processes. In eutherian mammals, this is accomplished by the corpus callosum, the largest tract in the brain. However, non-placental mammals, such as monotremes and marsupials lack a corpus callosum; instead, all neocortical interhemispheric connections cross the midline through the anterior commissure (ac). An important feature directing callosal development and function is the topographic arrangement of axons within the tract according to the place of origin of their cell bodies (Zhou *et al.,* (2013). *PNAS* 110; E2714-E2743). Here, we sought to determine whether spatial segregation of commissural axons predates evolution of the corpus callosum by studying commissural topography in dasyurid marsupials. We performed *ex-vivo* whole brain magnetic resonance high angular resolution diffusion imaging (HARDI) and tract-tracing with carbocyanines (DiI and DiD). HARDI Q-ball tractography revealed a parcellation of the ac carrying axons from olfacto-recipient structures, cingulate cortex and sensory neocortex. Moreover, tract-tracing experiments confirmed the results of tractographic reconstructions, by demonstrating a clear segregation of axons within the ac that corresponds to their place of origin. These results demonstrate that topography is conserved within commissures in marsupials and placentals, likely reflecting a feature conserved since their common ancestor. Our findings highlight an essential role for topographic axonal arrangement that may have been decisive during evolution of commissural wiring in mammals.

POS-THU-132

REGULATION OF TROPOMYOSIN RELATED KINASE-B RECEPTOR (TRKB) BY SUPPRESSOR OF CYTOKINE SIGNALLING (SOCS2)

Zamani A, Turnley A.M., Murray S
Department of Anatomy and Neuroscience, The University of Melbourne

Tropomyosin related kinase-B (TrkB) is a member of the tyrosine kinase family which, in response to its ligand Brain Derived Neurotrophic Factor (BDNF), can mediate pathways leading to neuronal survival and neurite outgrowth. It has been shown in our lab that SOCS2, known as a negative regulator of the JAK/STAT signalling pathway, affects neurite outgrowth by regulating EGF receptor signalling in PC12 cells and primary cortical neurons. In this study we hypothesised that SOCS2 may also have a regulatory role in neuronal function by regulating TrkB. Full length and mutated constructs of both TrkB and FLAG-tagged SOCS2 were transfected into HEK 293T cells to determine the regions of each protein required for interaction. Lysates were prepared and a protein pull down assay was done using anti-FLAG antibody. Immunoprecipitates and cell lysates were subjected to SDS-page followed by western blot using anti-Trk and anti-FLAG antibodies. SOCS2 interacted strongly with full length TrkB and weakly with TrkB mutants lacking the juxtamembrane or kinase domain respectively. No interaction was observed when both the juxtamembrane and kinase domains were deleted. This indicates that SOCS2 interacts with two intracellular sites in TrkB, possibly mediated by the SOCS2 SH2 and SOCS box domains and suggests a new role for SOCS2 as a regulator of BDNF signalling. This is a foundation for functional analyses in primary neurons and elucidation of the biological and cellular mechanisms involved. (229 words)

POS-THU-133

BIOPHYSICAL ANALYSIS OF SODIUM-POTASSIUM ATPASE MUTATIONS IDENTIFIED IN PATIENTS WITH ALTERNATING HEMIPLEGIA OF CHILDHOOD

Li MYS[1], Jazayeri D[1], Heinzen EL[2], Goldstein D[2], Petrou S[1]

1. Ion Channels and Disease, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia

2. Center for Human Genome Variation, Duke University, North Carolina, USA

Alternating hemiplegia of childhood (AHC) is characterized by sporadic paralysis and cognitive deficits. Recently, mutations in the ATP1A3 gene were identified in patients with AHC. It was observed that patients with E815K mutation have more severe symptoms than patients with D801N mutation. A current challenge is to explain this clinical heterogeneity, thus aid improving diagnosis and treatments.

ATP1A3 encodes for a subunit of the Na+/K+ ATPase which can be studied in Xenopus laevis oocytes. Activity of Na+/K+ ATPase was monitored by two-electrode voltage clamp. Data were presented as mean, statistical difference were tested with unpaired t-test, n=5-12 oocytes.

Forward cycling of Na+/K+ ATPase was activated with 15mM potassium, which resulted in an outward current of 94.9nA in wildtype. This outward current was similarly decreased in both AHC mutants (D801N 31.3nA; E815K 33.8nA). We next examined the proton leak of Na+/K+ ATPase and its dependence on sodium. In the absence of sodium, wildtype showed a proton leak of -86.5nA. While D801N showed similar proton leak (-70.8nA), the E815K mutant showed less proton leak (-30.1nA). In the presence of 50mM sodium, wildtype proton leak was decreased by 64.6%. A higher reduction was observed for D801N (80.7%). The proton leak of E815K was not altered by sodium.

While both AHC mutations resulted in profound loss of function in terms of the forward cycling, AHC mutants showed differences in proton permeability and sensitivity to sodium. This suggests that sodium and proton homeostasis potentially account for the clinical heterogeneity in AHC. (246 words)

POS-THU-134

SEGREGATED Aδ AND C-FIBER INFORMATION PATHWAYS THOUGH THE SOLITARY TRACT NUCLEUS.

Guo H & McDougall SJ
Florey Institute, University of Melbourne, Parkville, VIC, 3010.

Purpose: Viscerosensory afferents terminate at the solitary tract nucleus (NTS) within the brainstem. Viscerosensory neurons are Aδ or C-fiber afferents where each exhibits different activation and neurotransmission characteristics. C-fiber viscerosensory nerves express TRPV1 whereas Aδ nerves do not. Here we used a functional assay to map TRPV1 sensitive and insensitive pathways to NTS neurons. We determined if these pathway types converge at the second or higher order level within NTS or remain segregated. Methods: In slices incorporating the solitary tract (ST) and NTS, whole cell recordings of ST evoked PSCs were chartacterised in relation to shock intensity. Evoked ST-PCSs in the presence of capsaicin (100 nM) identified C-fiber pathways. Results: Graded intensity shocks activated ST axons and evoked postsynaptic currents (PSC) in NTS neurons (n=31). In most neurons responses became more complex with increasing shock intensity indicating convergent viscerosensory afferent input. Analysis of ST-PSC synaptic jitter differentiated monosynaptic from polysynaptic circuitry. Of second neurons receiving convergent monosynaptic input (n=10) or second order neurons receiving a combination of monosynaptic and polysynaptic input (n=11) and higher order NTS neurons receiving convergent polysynaptic input only (n=4), capsaicin either inhibited all or none of the activated pathways to each neuron. This indicates a complete segregation of TRPV1 sensitive and insensitive information pathways at the second through to the higher order level within NTS. Conclusion: The segregation of two broad information pathways throughout NTS indicates dedicated integration of Aδ and C-fiber related viscerosensory signals at the initial stages of central nervous system processing.

POS-THU-135

A POSTSYNAPTIC CONTRIBUTION TO SHORT-TERM SYNAPTIC PLASTICITY IN PIRIFORM CORTEX

Micallef AH, Stuart GJ

Eccles Institute of Neuroscience, Australian National University, Canberra ACT, Australia.

Short-term synaptic plasticity (STP) is a term used to describe activity-dependent changes in synaptic strength that last from seconds to minutes, and may play a role in short-term memory. STP is generally considered to be of presynaptic origin, and attributed to changes in the axon terminal. While less studied postsynaptic mechanisms also exist. This could occur due to changes in EPSP driving force or via activation of postsynaptic voltage-dependent mechanisms. Here we investigated the potential impact of these postsynaptic effects on the paired-pulse ratio (PPR) in the piriform cortex. Whole-cell recordings were made from semilunar and superficial pyramidal neurons in brain slices of rat piriform cortex, and EPSPs evoked by extracellular stimulation of the lateral olfactory tract or at the border of layer 1/2. Bath application of a low concentration of the AMPA receptor antagonist DNQX (2 µM) was used to reduce the amplitude of EPSPs via a purely postsynaptic mechanism. The PPR was measured before and after DNQX application. Application of DNQX increased the PPR from 0.92 ± 0.17 to 1.68 ± 0.23 (n=13; $p < 0.001$), indicating a switch from depression to pair-pulse facilitation. Furthermore, a positive correlation was observed between EPSP size and the change in the PPR after DNQX application ($R^2 = 0.38$, $p < 0.01$). Overall, the data are consistent with the idea that postsynaptic changes in either electrical driving force or voltage-dependent mechanisms play a role in determining the sign and magnitude of short-term plasticity in piriform cortex. (244 words).

POS-THU-136

METHYLPHENIDATE AMPLIFIES LONG-TERM POTENTIATION IN CA1 OF THE RAT HIPPOCAMPUS THROUGH β-ADRENERGIC AND D_1/D_5 RECEPTORS.

Morales B[1], Contreras D.[1], Carreño M.[1], Carvallo C[1], Zeise M [2],
[1]Depto. de Biología, Fac. Química y Biología, Universidad de Santiago de Chile, [2]Escuela de Psicología, Fac de Humanidades, Universidad de Santiago de Chile.

Methylphenidate (MPH) is a psychostimulant used in the therapy of the Attention Deficit/Hyperactivity Disorder and recently also has become a drug of abuse. Our and other laboratories have demonstrated that MPH modifies synaptic plasticity in the hippocampus. However, as understanding of the cellular and molecular mechanisms involved is still incomplete, we investigated the effects of MPH on LTP in hippocampus slices. 3-4 weeks old Sprague-Dawley rats were decapitated under halothane anesthesia, and hippocampus slices (400 µm thick) were prepared. LTP was induced in the CA1 region by applying theta burst stimulation (TBS, 5 trains, 100Hz) at the Schaeffer collaterals. Superfusion during 20 min with MPH, increased the magnitude of LTP in a dose-dependent manner (from 134.6±1.2 % (controls) to 137.5 ± 2.8 7% (3 nM; n=3,3; $p>0.05$), 163.4±10.4% (50 nM; n=5,7; $p<0.05$), 194.3±5.8 (5 µM; n=6,8, $p<0.01$), and 196.4±4.2% (50 µM; n=4,4; $p<0.01$). The paired-pulse curves remained unchanged after perfusion with MPH, suggesting that the effect of MPH does not involve modifications of presynaptic components. The increase induced by MPH was inhibited by 5µM timolol, a β-adrenergic blocker, from 194.3±5.8% (TBS+5µM MPH) to 152.7±1.66% (TBS+5µM MPH+Timolol; n=4,4; $p<0.01$). Interestingly, LTP increase was also inhibited by 5 µM of SCH23390, a D1/D5 blocker, from 192±7% (TBS+5µM MPH) to 151.3±0.9% (TBS+MPH+SCH23390; n=3,3; $p<0.01$). Only this last effect is postsynaptic because the paired-pulse curves remain unchanged. These results suggest that MPH increases LTP in a dose-dependent manner involving β-noradrenergic and D_1/D_5 receptors through a polysynaptic mechanism.

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POS-THU-137

THE MAKING OF THE NEUROMOTOR SYSTEM: THE ROLE PLAYED BY GLYCINERGIC CONNECTIONS ONTO DEVELOPING MOTOR NEURONS.

Peter G. Noakes [1,2]· Matthew J. Fogarty[1], Refik Kanjhan[2] and Mark C. Bellingham[2] [1]School of Biomedical Sciences, [2] Queensland Brain Institute, University of Queensland Qld 4072, Australia.

The number of motor neurons (MNs) innervating a given muscle depends on nervous system activity as well as feedback from its muscle. We propose that Glycinergic inputs onto developing MNs act to regulate its development. 300 µm brainstem slices from wild- type mice and mice lacking gephyrin were used to record synaptic currents from hypoglossal (XII) MNs, that post recording were filled with Neurobiotin by electroporation. These slices were then immuno-stained for the location and distribution of excitatory glutamergic and inhibitory GABAergic synapses. Anti-VGLUT2 and anti-PSD95 located glutamergic synapses. Anti-VGAT and anti-GABAα1 located GABAergic synapses. MN morphology and synapses were assessed from serial confocal images subjected to IMARIS software processing (Fogarty et al., 2013). In gephyrin-/- mice, a model of perturbed glycinergic transmission, XII MNs responded by increasing the length of their dendritic branches. Along these dendrites, the number of dendritic spines and filopodia were also increased. Analyses of synaptic number revealed that the number of excitatory glutamergic synapses but not GABAergic synapses increased in XII MNs from gephyrin-/- mice. Electro-physiological analyses showed increased in excitatory post-synaptic potentials and frequency of action potential generation in XII MNs from gephyrin -/- mice. This suggests that XII MNs became more active in the absence of glycinergic transmission. This enhanced activity resulted from the increased number of glutamergic synapses on XII MN distal dendrites in gephyrin-/- mice. This increased activity was not compensated for by corresponding increases in GABAergic synapses.
Fogarty et al., 2013 Frontiers in Neural Circuits 7: Article 153.

POS-THU-138

BIOGENESIS, COMPOSITION AND MORPHOLOGY OF SECRETORY VESICLES IN THE LYSOSOMAL STORAGE DISORDER MUCOPOLYSACCHARIDOSIS IIIA.

Hocking K[1], Brooks DA[1], Keating DJ[2], Parkinson-Lawrence EJ[1].

1. Mechanisms in Cell Biology and Disease Research Group, School of Pharmacy and Medical Science, Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia. 2. Molecular and Cellular Neuroscience Group, Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, Australia.

Mucopolysaccharidosis IIIA is a neurodegenerative lysosomal storage disorder resulting from a decrease in the lysosomal hydrolase sulphamidase. Although neuropathology is the major clinical manifestation in MPS IIIA patients, there is little understanding of the functional event(s) that lead to this pathology.

In this study we have characterised the secretory granules in MPS IIIA mouse chromaffin cells and the ultrastructure of the presynaptic terminals in MPS IIIA mouse neurons, in comparison to controls. In MPS IIIA mouse chromaffin cells there was no change in VAMP-2 and synaptophysin, but a significant increase in VAMP-4, a marker of immature granules. Interestingly, there was a relative decrease in the general granule marker synaptotagmin-1, which is involved in calcium sensing and secretory vesicle docking. In MPS IIIA presynaptic terminals we observed a significant increase in the average length of synapses, increased numbers of synaptic vesicles and a population of aberrantly large vesicles, together with a reduction in mitochondria. These changes in the biogenesis, composition and morphology of secretory vesicles, together with reduced potential for energy utilisation, may contribute to the impaired neurotransmission in MPS IIIA. These novel findings provide a potential link between lysosomal storage and the observed neurological dysfunction in MPS IIIA patients. (199 words).

POS-THU-139

INVESTIGATION OF THE INTRACELLULAR CALCIUM SIGNALING CASCADES MEDIATED BY NCAM2 DURING NEURONAL DIFFERENTIATION USING GENETICALLY ENCODED CALCIUM REPORTERS.

Sheng L, Leshchyns'ka I, Sytnyk V.

School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia.

Cell adhesion molecules serve pivotal functions in brain development by modulating Ca^{2+} levels in growing neurons. Changes in Ca^{2+} concentration play an important role in regulation of a number of processes in growing neurons, including cytoskeleton remodelling and gene expression. Neural cell adhesion molecule 2 (NCAM2) regulates brain development. The downstream Ca^{2+} signaling pathways mediated by NCAM2 however have remained unknown. In our work, we have used new genetically encoded fluorescent reporters containing subcellular localization signals to monitor Ca^{2+} levels in growing neurons at high subcellular resolution. We show that these reporters are correctly targeted to different subcellular compartments in neuronal cell lines and primary cortical neurons, and are suitable to detect Ca^{2+} changes induced by spontaneous activity and Ca^{2+} influx via voltage gated Ca^{2+} channels. By using these reporters in live cell imaging experiments, we show that Ca^{2+} influx via voltage gated Ca^{2+} channels induces Ca^{2+} changes not only in the cytosol but also in the nucleus of neurons. To investigate the relationship between NCAM2 activation and downstream intracellular Ca^{2+}-signaling cascades, we used NCAM2 antibodies to cluster and activate NCAM2 at the cell surface membrane. In cultured cortical neurons, application of NCAM2 antibodies induced transient increase in submembrane [Ca^{2+}], which occasionally induced increase in cytosolic [Ca^{2+}]. We propose that NCAM2-mediated modulation of the intracellular Ca^{2+} levels plays a role in regulation of neuronal morphology. (225 words).

POS-THU-140

DIFFERENTIAL INHIBITORY INPUT TO CALRETININ EXPRESSING NEURONS IN THE MOUSE SUPERFICIAL DORSAL HORN

KM Smith[1], JF Madden[1], SA Dickinson[1], KA Boyle[2], DI Hughes[2], RJ Callister[1], P Jobling[1] and BA Graham[1]

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle and Hunter Medical Research Institute (HMRI), [2] Institute of Neuroscience and Psychology, and College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Substantial neuronal diversity exists within the spinal dorsal horn (DH), a key region where peripheral sensory information is processed. This heterogeneity makes identification and study of discrete populations difficult. Purpose: To characterise the inhibitory input to calretinin (CR) containing dorsal horn neurons. Method : Transgenic mice (n=9) expressing enhanced green fluorescent protein in calretinin positive neurons were anesthetised (ketamine 100mg/kg) and parasagittal spinal cord slices (250µm) were obtained. Using a CsCl-based internal solution containing neurobiotin, we made targeted patch-clamp recordings of inhibitory postsynaptic currents (IPSCs). Results: Our morphological analysis differentiated two CR-positive neuron classes: those with islet features (n=11); and a more diverse group including radial (n=15), vertical (n=1), and central (n=1) morphologies (non-islet). Our recordings showed that IPSC frequency, amplitude and decay time constant were similar in islet and non -islet populations. In contrast, IPSC rise times were significantly slower in islet recordings (2.1 ± 0.2 vs 1.5 ± 0.1ms, p=0.009). Analysis of amplitude-rise time relationships also detected a correlation in islet (r^2=0.44), but not non-islet recordings (r^2=0.12) implying greater electrotonic filtering.

Finally, input resistance was lower in islet versus non-islet cells (904±100 vs 1397±162MΩ, p=0.023). Conclusion: Two CR-positive DH neurons populations exist with distinct morphological, biophysical and inhibitory synaptic properties. In islet cells the lower input resistance is consistent with larger somatodendritic arbours, and slower IPSC rise times and enhanced electrotonic filtering suggest more distally located inhibitory synapses. Together these data indicate distinct inhibitory regulation of these two populations with likely implications for each in spinal sensory processing.
(248 words).

POS-THU-141

ROLE OF TLR2/4 RECEPTORS IN MORPHINE-INDUCED INHIBITION OF MURINE DISTAL COLON

Staikopoulos V[1], Beckett EAH[1], Hutchinson M[1]

[1]Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, SA 5005.

Toll-like receptors (TLRs) are expressed in enteric neurons, glia, gastrointestinal (GI) smooth muscle and mucosal epithelial cells, yet the functional role of these receptors within the GI tract remains unclear. TLR receptors mediate many of the undesirable central effects of chronic opioid administration including opioid-induced hyperalgesia and dependence, via activation of central microglia. Clinically, opioid-induced constipation remains one of the main reasons for reduced administration or withdrawal of opioid analgesics. Here, the role of TLR2/4 receptors in the morphine-induced inhibition of colonic motility was investigated *in vitro* using intact colon preparations and isolated distal colon segments from Balb/c wild-type and TLR2/4 knockout (KO) mice. 0.1µM morphine abolished colonic migrating motor contractions (CMMCs) at the distal end of intact wild-type colons, whereas 1µM morphine was required to abolish CMMC activity in distal TLR2/4 KO colon, suggesting a rightward shift in morphine sensitivity with the loss of TLR2/4 receptors. Electrical field stimulation (0.5ms pulses; 5-10Hz; 10s trains) applied to the mid-region of intact colon preparations evoked small amplitude circumferential relaxations of both wild-type and TLR2/4 KO distal colon. In intact colon preparations, morphine (1µM) potentiated the amplitude of distal colon relaxations in wild-type but not in TLR2/4 KOs. In isolated, precontracted segments of distal colon, EFS elicited longitudinal axis relaxations, which were potentiated by morphine in wild-type, but not in TLR2/4 distal colon. These data suggest that TLR2/4 receptors and/or their downstream pathways may contribute to the inhibitory effects of morphine in the colon.

POS-THU-142

MOLECULAR MECHANISMS OF ABNORMAL NEUROTRANSMISSION INDUCED BY DISRUPTION OF NCAM-MEDIATED SYNAPTIC ADHESION IN CNS SYNAPSES

Leshchyns'ka I, Sytnyk V
School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

The neural cell adhesion molecule (NCAM) is a member of the immunoglobulin superfamily of cell adhesion molecules. Abnormalities in expression and proteolysis of NCAM have been observed in schizophrenia and bipolar disorder and were suggested to affect neurotransmission in the brain via mechanisms, which remain poorly understood. We show that NCAM accumulates in the presynaptic boutons of active excitatory glutamatergic synapses. We also demonstrate that NCAM promotes maturation of the synaptic vesicle recycling machinery by recruiting to the presynaptic membrane the adaptor protein 2 (AP-2), a protein complex involved in clathrin-dependent synaptic vesicle endocytosis. Disruption of the NCAM-mediated synaptic adhesion results in reduced synaptic vesicle recycling and inhibits the ability of synapses to respond to the repetitive stimulation. Our results thus indicate that disruption of NCAM functions in humans may result in abnormalities in neurotransmitter release, which may contribute to aetiology of psychiatric disorders. (178 words).

POS-THU-143

EPHA4 MODULATES HIPPOCAMPAL NEUROGENESIS AND SYNAPTIC PLASTICITY IN THE ADULT MOUSE

Taylor CJ, Newcombe EA, Turpin F, Boyd AW, Sah P, Bartlett PF

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

EphA4 has been shown to regulate neurogenesis in the developing brain, as well as adult neurogenesis in the subventricular zone and cortex. Furthermore, EphA4 has been demonstrated to regulate synaptic plasticity in the CA1 region *in vitro*. We were therefore interested to investigate whether EphA4 regulates neurogenesis and synaptic plasticity in the dentate gyrus of the adult mouse. Using the neurosphere assay, we found that hippocampal stem and precursor cells cultured from EphA4$^{-/-}$ and EphA4 kinase-dead (EphA4$^{KD/KD}$) transgenic mice produce a significantly greater number of neurospheres compared to wild-type controls, indicating that EphA4 modulates proliferation of stem and precursors in the adult subgranular zone. Mice unilaterally implanted for acute *in vivo* electrophysiology, in which perforant path-evoked synaptic field potentials were recorded from the dentate gyrus, received high-frequency stimulation (HFS) to induce long-term potentiation (LTP). EphA4$^{-/-}$ and EphA4$^{KD/KD}$ mice showed significantly reduced LTP two hours after HFS, compared to wild-type controls. This deficit could be completely restored by intraperitoneal administration of the NMDA receptor co-agonist D-serine, without affecting the magnitude of LTP in control mice. We are currently investigating the involvement of the NMDA receptor in mediating these two observations, that EphA4 modulates both adult hippocampal neurogenesis and synaptic plasticity. (222 words)

POS-THU-144

SPONTANEOUS CA^{2+} TRANSIENTS IN THE NERVE TERMINAL OF NEOCORTICAL PYRAMIDAL NEURONS

Tran L.T.V.[1], Stricker C.[1,2]

[1] Eccles Institute of Neuroscience, The John Curtin School of Medical Research, The Australian National University (ANU), Canberra, ACT 2601, Australia. [2] ANU Medical School, Canberra, ACT 2601, Australia.

Recent studies have suggested that spontaneous neurotransmission can arise from the release of Ca^{2+} from presynaptic stores via the ryanodine and/or inositol 1,4,5-trisphosphate receptors. However, direct evidence for the role of intracellular Ca^{2+} stores in spontaneous transmitter release at excitatory synapses is lacking. Objective: To image and characterise spontaneous Ca^{2+} transients in the nerve terminal of neocortical pyramidal neurons. Methods: Layer V pyramids in the rat somatosensory cortex are filled with Alexa 568 (50µM) and Oregon Green 488 BAPTA-1 (OGB-1, 80µM) for > 30min. Synaptic boutons, identified along $1^{st}/2^{nd}$ order axon collaterals using Alexa 568 fluorescence, are imaged and verified to be release sites based on the fluorescence transient of OGB-1 in response to an AP triggered at the soma. OGB-1 fluorescence across the diameter of the terminal is then monitored by line scanning at 10–20 Hz for a total duration of ~15min per bouton, in 3min recording periods. Results: As predicted, spontaneous Ca^{2+} transients are observed at low frequency (0.005 ± 0.002 Hz, *n* = 13 boutons – 8 cells) in 1µM TTX. These transients appear to be highly variable in amplitude and often occur in bursts: ~60% of all transients take place within 5s of each other, occasionally summating to produce a large and extended rise in the intracellular Ca^{2+} concentration. Conclusion: We have, for the first time, detected spontaneous Ca^{2+} transients in the nerve terminal of neocortical pyramidal neurons. Although to be verified, these transients most likely stem from the release of Ca^{2+} from presynaptic Ca^{2+} stores. (250 words)

POS-THU-145

EFFECTS OF ENDOGENOUS OPIOIDS ON THE ACTIVITY OF INTERCALATED NEURONS OF THE AMYGDALA: IMPLICATIONS FOR ANXIETY DISORDERS.

Winters BL, Bagley EE

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

Neural circuits in the amygdala are key mediators of fear memory acquisition and storage, but also act to reduce the fear response once the threat has passed (extinction). Anxiety disorders, such as post-traumatic stress disorder, may result from impaired extinction of fear memories. Extinction of the fear response relies on a group of GABAergic interneurons in the amygdala, the intercalated cells (ITC). Intriguingly, it has been reported that endogenous opioids play a role in modulating fear memory. Indeed, knockout mice negative for enkephalin, an endogenous opioid highly expressed in ITCs, display anxious, fearful and aggressive behaviour. However, the effects of opioids on ITC activity are unknown. Using whole-cell, voltage-clamp electrophysiology, we show that exogenous application of met-enkephalin (ME) reduces the amplitude of $EPSC_{AMPA}$ (60 ± 2%, n =12) at basolateral amygdala (BLA)-ITC synapses. Similarly, ME also reduced $IPSC_{GABA}$ (90 ± 0.6%, n = 8) at local ITC-ITC synapses. In both cases, the paired-pulse ratio was increased, suggesting a decrease in neurotransmitter release from presynaptic sites. Further, following modest field stimulation (5 stimuli at 150 Hz) the opioid antagonist naloxone increases $EPSC_{AMPA}$ at BLA- ITC synapses (24.2 ± 0.2 %, n = 4) indicating that endogenous opioids are present and acting to reduce glutamate release. These data suggest that endogenously released opioids are significant modulators of amygdala function and may participate in regulating the extinction of fear memories. (228 words).

POS-THU-146

INVESTIGATING THE ROLE OF TLR4 IN THE SYNERGISTIC INTERACTION OF ETHANOL AND BENZODIAZEPINE

Lagiseti R, Corrigan F, Hutchinson MR.

Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, Australia.

Co-administration of ethanol (alcohol) and benzodiazepine commonly occurs for the enhanced euphoric and sedative effects. Increased incidence of fatal overdose implies a synergistic, or super-additive, effect of this drug combination. This synergistic effect has been characterized by dose-response using the sleep time test in mice as a measure of sedation.

Central immune activation has previously been found to influence behaviour. TLR4 receptors are present on glia in the CNS and recognise a variety of molecular patterns including some drugs, causing pro-inflammatory cytokine release that modulates neuronal activity and thereby some behaviours. Given the established role for TLR4 mediating the synergistic interaction of alcohol and morphine, it is hypothesized that TLR4 may serve as a novel site of alcohol-benzodiazepine interaction. Using genetic null mutant mice administered a combination of alcohol and benzodiazepine TLR2/4 knockout mice displayed significantly shorter loss of righting reflex (LORR) than wild type in response to diazepam plus alcohol ($p < 0.05$). However, inhibition of NF-κB and IL1 receptor, which are downstream effectors of the MyD88 signalling pathway, did not mitigate sedation ($p < 0.05$). Analysis of glial reactivity, pro-inflammatory markers, and examination of early response genes will be used to assess acute neuroimmune consequences of this drug-drug interaction. TLR4-mediated synergy would provide a new avenue of investigation for the treatment of potentially fatal benzodiazepine plus alcohol overdoses by administering known TLR4 blockers. (225 words).

POS-THU-147

MONOCARBOXYLATE TRANSPORTER 8 IS EXPRESSED ON OLIGODENDROCYTE PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS.

Lee JY[1], Kim MJ[1], Stanley EG[1,3], Elefanty AG[1,3], Petratos S[1,2].

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia;

[2]Central Clinical School, Monash University, Prahran, Victoria, 3004, Australia;

[3]Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

Thyroid hormone plays a significant role in brain development, responsible for critical cell cycle events during oligodendrogenesis. Specifically, triiodothyronine (T3) is an essential regulator of terminal oligodendrocyte differentiation and myelination in vivo. Transport of thyroid hormone into neural cells is not simply a passive diffusion process but rather an active process involving specific thyroid hormone transporters. The monocarboxylate transporter 8 (MCT8) has recently been defined as a T3-specific transporter that is crucial for brain development. In humans, *mct8*-deficiency results in the X-linked-inherited psychomotor retardation disorder, known as Allen-Herndon-Dudley syndrome (AHDS). Because dysmyelination is a feature of this disease, we hypothesised that MCT8 may play a significant role in oligodendrocyte development and myelination. We have established a novel method of derivating pre-myelinating O4-positive oligodendrocytes from human embryonic stem cell (hESC) lines by utilising either the NKX2.1-GFP or PAX6-mCherry as reporters. By live cell imaging (n=5) and flow cytometry (n=5), expression profile of NKX2.1-GFP at the early differentiation stage was determined and GFP+ and GFP- cells were separated by flow-activated cell sorting (FACS). By immunocytochemistry, flow cytometry and quantitative polymerase chain reaction (qRT-PCR), GFP+ cells differentiated towards OPCs (CD140a+/NG2+) then immature oligodendroglia (O4+) whereas GFP- cells differentiate towards neurons (βIII-tubulin+). From these cells, we have demonstrated that MCT8 is expressed on subsets of oligodendrocyte progenitor cells (OPCs), and immature oligodendroglia. Our results highlight the possible role of MCT8 in thyroid hormone transport for oligodendrocyte development and may implicate this transporter as a central co-determinant in the promotion of myelinating oligodendrocyte.

POS-THU-148

THE ROLE OF OESTROGENIC INNATE IMMUNE PRIMING IN EXACERBATED FEMALE PAIN

Nicotra L1, Watkins L2, Tuke J3, Kwok H1, Rolan P1, Hutchinson MR4.

1. Discipline of Pharmacology, School of Medical Sciences, The University of Adelaide, Adelaide, Australia. 2. Center for neuroscience, The University of Colorado, Boulder, Colorado, USA. 3. School of Mathematical Sciences, The University of Adelaide, Adelaide, Australia. 4. Discipline of Physiology, School of Medical Sciences,The University of Adelaide, Adelaide, Australia.

Chronic pain is a world-wide epidemic problem. Females succumb to this debilitating condition more frequently than men, with sex steroids implicated in the increased pain prevalence. The recent acceptance of innate immune involvement in chronic pain and an interaction between the sex steroids and the innate immune system opens a new avenue to explore sex differences in chronic pain (Nicotra et al., 2012). This study aimed to investigate the role of 17β-oestradiol innate immune cell priming in exacerbated female pain. Male and female mice underwent nerve injury utilising the Grace model of graded neuropathy and allodynia was assessed using von Frey, with oestrus phase assessed each test day. Glial modulating agents were assessed in ovariectomised and oestrogen supplemented mice. Novel approaches such as adoptive transfer of female pain phenotype to males by transferring female microglia allowed for the determination of oestrogen primed microglia in female chronic pain. Differences between treatments and sexes were analysed with RStudio using repeated measures linear mixed effects modelling. Post nerve injury, females were significantly more allodynic than males, with exacerbated pain at phases of high oestrogen. Greater pain reversal was demonstrated utilising glial modulating agents in female and oestrogen supplemented mice. Furthermore, intrathecal transfer of activated female microglia alone induced greater mechanical hypersensitivity, with oestrogen primed microglia found to exaggerate pain. This study significantly advances the knowledge of female chronic pain mechanisms. Our discoveries highlight greater potency of neuroimmune targeted therapies in females, suggesting female chronic pain should be treated differently from that in males. (250 words).

POS-THU-149

FYN KINASE IS NECESSARY FOR BDNF TO PROMOTE OLIGODENDROCYTE MYELINATION IN VITRO.

Peckham HM[1], Giuffrida L, Wong AW, Ferner AH, Kilpatrick TJ, Murray SS, Xiao J

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

Background Using an *in vitro* model of myelination, we have previously shown that BDNF acts via oligodendrocyte-expressed TrkB receptors and the mitogen-activated protein kinases Erk1/2 to promote myelination. Interestingly, an unrelated Src family kinase, Fyn, is required for oligodendrocyte differentiation *in vitro* and myelination *in vivo*. We hypothesised that Fyn is an intermediate kinase that BDNF utilises to activate Erk.

Results Here we show that BDNF stimulates Fyn autophosphorylation (n=3) in myelinating co-cultures. This is a TrkB dependent effect, as shRNA-mediated knockdown of TrkB in oligodendrocytes prevents Fyn autophosporylation. Importantly, the promyelinating influence of BDNF is abrogated in the presence of PP2, a pharmacological inhibitor of Src family kinases (n=3). In order to specifically interrogate the effect of Fyn in oligodendrocytes we virally manipulated oligodendrocytes to overexpress wild type (WT) or kinase dead (KD) Fyn and used these oligodendrocytes in the *in vitro* myelination assay. The expression of KD Fyn attenuated the BDNF-induced activation of Erk1/2 (n=2) and inhibited myelination as assessed by a reduction in the number of myelinated axonal segments (n=3) and reduction in the expression of myelin proteins (n=1). In contrast, the expression of WT Fyn promoted myelination as assessed by an increase in the number of myelinated axonal segments (n=3) and increase in the expression of myelin proteins (n=1)

Conclusions Collectively these data suggest that that BDNF activation of oligodendroglial TrkB receptors stimulates phosphorylation of Fyn, which leads to Erk activation and stimulation of the myelin program.

POS-THU-150

HYPERMETHYLATION OF MICRORNA-16 IS TUMOR SUPPRESSIVE IN GLIOMAS AND GLIOMA STEM CELLS

Zhengnan Shan 1, Feng Liu 2, Xiaoyuan Liu 2 , Lihua Li 2,Jie He3

1 School of Pharmacy and Medical Sciences, Faculty of Health Sciences, University of South Australia, Adelaide, SA, Australia
2 Wuxi Tumor Research Institute, The Fourth Affiliated Hospital of Soochow University
3 Department of Pathology, The Affiliated Provincial Hospital of Anhui Medical University
*Corresponding author: Dr. Jie HE, Email: hejie2009wuxi@gmail.com

MicroRNAs (miRNAs) are small RNA (~22 nt), noncoding RNAs that negatively regulate target gene expression. Some miRNAs that function as tumor suppressors could be inactivated by aberrant hypermethylation and modification of histones. Study showing that miR-16 is down-regulated in gliomas and up-regulated in glioma stem cell. In this study, several epigenetic alterations were identified in regulating miR-16 expression and function in carcinogenesis of gliomas.

MiR-16 expression was tested by RT-qPCR in glioblastoma cell lines (GCLs) and glioma stem cells (GSCs) treated with 5-aza-2'-deoxycytidine (5-Aza-CdR, DNA methyltransferases inhibitor) and 4-phenylbutyric acid (PBA, histone deacetylase inhibitor). The CpG islands (CGI) methylation of miR-16 was determined by Bisulfite Sequencing PCR in GCLs and GSCs. Cell proliferation, apoptosis, invasive were estimated respectively. Based on preliminary results, four CGI methylation of miR-16 have been identified in GCLs and GSCs. The epigenetic drugs 5-Aza and PBA differentially up-regulate expression of miR-16 in GCLs and GSCs. Overexpression of miR-16 decreased the proliferation and invasive ability of GSCs. In addition, the expression on account of miR-16 in 5-Aza and PBA treated reduced self-renewal and clonogenic survival in GSCs. Our results suggest that the re-expression of miR-16 due to epigenetic drugs suppresses the growth of GCLs and GSCs. This finding may offer new biomarkers and therapeutic targets in gliomas. Project is funded by national natural science foundation of China, No.81272800.

POS-THU-151

NON-STEREOSELECTIVE OPIOID BINDING IN THE MOUSE BRAIN.

Thomas J[1], Somogyi AA[1], Hutchinson MR[2].

[1]. Discipline of Pharmacology, University of Adelaide, Australia. [2]. Discipline of Physiology, University of Adelaide, Australia.

In the 1960's when opioid research was in its infancy, attention was focused directly toward the stereoselective neuronal receptors that were discovered to be critical for opioid analgesic responses. However, opioids can paradoxically increase pain sensitivity in humans and rodents leading to hyperalgesia (heighted pain) and analgesic tolerance. Recent discoveries have identified both neuronal and neuroimmune mechanisms that contribute to altered opioid action. Our previous work has demonstrated that co-administration of the opioid receptor inactive stereoisomer (+)-naloxone significantly reduces the development of hyperalgesia and tolerance to opioids, through diminished neuroinflammatory responses. Thus, opioid agonists are thought to bind both stereoselective neuronal opioid receptors that produce analgesia, and to non-stereoselective receptors that are responsible for the neuroinflammatory response leading to compromised opioid analgesia and various unwanted actions. However, existing data on the kinetics of non-stereoselective opioid binding is limited and requires further investigation. Balb/c mouse brains were harvested and homogenised using the gentleMACS™ Octo Dissociator. Homogenates were diluted to 10mg/mL protein and $^{[3H]}$(-)-naloxone added to 1.63nM. $^{[3H]}$(-)-Naloxone dissociation was initiated by adding 1µM of cold drug. 1mL aliquots were vacuum filtered and washed with 10mL of cold 0.9% PBS at required time points. 30.3% (+/- 4.9%; $P<0.01$) of (-)-naloxone specific binding was displaced by (+)-naloxone in Balb/c brain homogenates. These results emphasise the extent of the non-stereoselective receptor in (-)-naloxone binding and its potential to modify opioid action.

POS-THU-152

MACROPORE FORMATION VIA P2X7 AND P2X4 RECEPTORS IS BLOCKED BY MINOCYCLINE IN PRIMARY MICROGLIA AND A HETEROLOGOUS EXPRESSION SYSTEM.

Watson, JM Stokes, L. Horton, A. Stebbing, MJ. School of Medical Sciences, HIRI, RMIT University, Bundoora, Victoria 3083

Purpose: Microglia become activated in response to various stimuli including ATP released from damaged neurons and act as immune cells causing inflammation. Inappropriate activation of microglia has been implicated in various CNS pathologies. The drug minocycline can inhibit microglial activation in vivo. We have previously shown it can block an early event in microglial activation, namely formation of membrane pores (macropores) that admit large molecules eg. DNA binding dyes, but its mechanism of action is not clear. We therefore investigated minocycline's ability to block P2X receptors, which have been implicated in macropore formation.

Methodology: Rat microglia isolated from neonatal mixed glial cultures or HEK- 293 cells transfected with either rat P2X4 or P2X7 receptors were placed in Kreb's HEPES buffer and treated with ATP.Uptake and fluorescence of Yo-Pro1 was monitored using confocal microscopy (microglia) or a Flexstation3 plate reader (HEK-293 cells).

Results: In response to 1mM ATP, dye uptake indicating macropore formation occurred in 44% of microglia tested (n=71) and was inhibited by either minocycline (100uM) or P2X7 antagonist AZ10606120 (10uM). Similarly, dye uptake into HEK-293 cells stably transfected with P2X7 was reduced to 52% of control by minocycline (100uM, n=16 wells, 3 independent experiments, $p<0.01$, ANOVA) and completely blocked by AZ10606120 (10uM). Dye uptake in response to 50uM ATP in HEK-293 cells transiently transfected with P2X4 was reduced by minocycline (100uM) to 45% of control (n=6 wells, 2 transfections,$p<0.01$ ANOVA).

Conclusion: Minocycline appears to act directly on both rat P2X4 and rat P2X7 receptors to block ATP-induced macropore formation. (250 Words)

POS-THU-154

INVESTIGATION OF THE INVOLVMENT OF CALCIUM REGULATION IN THE UNILATERAL ROTENONE-LESIONED MOUSE MODEL OF PARKINSON'S DISEASE

Rcom-H'Cheo-Gauthier AN, Meedeniya ACB, Pountney DL

Griffith Health Institute, School of Medical Science, Griffith University, Gold Coast, Queensland, Australia.

Introduction: Neurodegeneration in Parkinson's disease is associated with protein aggregation and the formation of neuronal Lewy bodies mainly composed of the protein alpha-synuclein. Alpha-synuclein has been shown to aggregate when intracellular calcium levels are elevated. Moreover, the calcium buffering protein, Calbindin, the expression of which results in relative sparing of Calbindin-positive neurons in PD, may provide a degree of protection against the pathological process in Parkinson's disease, pointing to the influence of calcium dysregulation in neurodegeneration. Methods: Unilateral lesioning of the medial forebrain bundle of C57 black mice with the mitochondrial inhibitor, rotenone, was performed to induce oxidative stress and stimulate neurodegeneration. Fluorescence immunohistochemistry of brain tissue was used to determine the frequency of Calbindin-28K positive cells and alpha-synuclein aggregates. Results: Confocal microscopy of mouse brain tissue sections showed more frequent Calbindin-28K positive cells within the lesioned hemisphere (p, 0.05) than within the control hemisphere. The data indicates more frequent alpha-synuclein aggregates within the lesioned hemisphere, and that aggregates are less numerous in Calbindin-positive cells. Conclusion: These findings suggest an association between the calcium buffering protein, Calbindin-28K, and neuronal survival and alpha-synuclein aggregation in a mouse model of Parkinson's disease and implicate the involvement of calcium dysregulation in Parkinson's disease. (202 words).

POS-THU-155

p75NTR EXTRACELLULAR DOMAIN AS A BIOMARKER IN MOTOR NEURON DISEASE

Shepheard SR[1], Schultz D[2], Chataway T[1], Rush RA[1], Rogers M-L[1]

1. Dept. Human Physiology & Centre for Neuroscience, Flinders University, Adelaide, South Australia, Australia. 2. Dept. Neurology, Flinders Medical Centre, South Australia, Australia.

Objective biomarkers for Motor Neuron Disease (MND) that reflect disease progression are essential to allow screening of potential new treatments. Biomarkers may also help detect disease early in people with genetic (familial) mutations leading to MND. Urine as a source of biomarkers should be investigated. Neurotrophin receptor p75NTR re-expression after nerve injury and cleavage of its extracellular domain ($p75NTR^{ECD}$) is part of a homeostatic program removing defective neurons after injury. We have previously found $p75NTR^{ECD}$ in urine of MND patients and now evaluate quantitative estimation of urinary $p75NTR^{ECD}$ as an objective biomarker for MND.

A quantitative sandwich ELISA (sELISA) was used to detect $p75NTR^{ECD}$. Urine and neurological data were collected from 28 patients with MND, 9 with Parkinson's disease (PD), 10 with Multiple Sclerosis (MS) and 12 age-matched, healthy controls. $p75NTR^{ECD}$ was also measured multiple times from 14 MND patients.

Urinary $p75NTR^{ECD}$ levels measured by sELISA predict MND from healthy controls. The mean value of urinary $p75NTR^{ECD}$ from MND patients was significantly higher (7.9±0.5ng/mg creatinine, $p<0.001$) than healthy individuals (2.6±0.2ng/mg creatinine) and patients with other neurological conditions (4.1±0.2ng/mg creatinine PD & MS). MND patients with faster disease progression had higher mean $p75NTR^{ECD}$ levels than those with slower progressing disease ($p=0.004$).

These findings indicate urinary $p75NTR^{ECD}$ offers utility as a biomarker of MND and may be useful in predicting disease severity. Further investigation will include measurement of $p75NTR^{ECD}$ levels in blood and in familial MND patients prior to the onset of symptoms. (241 words).

POS-THU-156

CHARACTERISATION AND TARGETING OF P75NTR EXPRESSING MOTOR NEURONS IN SOD1G93A MICE

Smith KS[1], Rush RA[1] and Rogers ML[1]

1. Centre for Neuroscience, Department of Human Physiology, Flinders University, GPO Box 2100, Adelaide, SA, 5001

This study investigated changes in motor neurons of MND (SOD1G93A) mice, including re-expression of the neurotrophin receptor p75 (p75NTR). SOD1G93A mice and age matched controls (B6) at 60d, 80d, 100d, 120d, end-stage-145days; (n=4/age/strain) had the lumbar region removed after perfusion fixation, sectioned and analysed for p75NTR, ChAT, and ATF-3. Fluorescently labelled anti-p75NTR antibody, MLR2 (MLR2-488), was injected IP into 4 SOD1G93A at 100 to 110 days and examined histologically. Motor neurons identified by ChAT have a significantly smaller average size in affected mice compared with the B6 controls from 100 days onwards ($p<0.05$). The SOD1G93A motor neurons also shrink as the mouse ages, with a significant difference in size distribution compared to B6 controls from 80 days onward ($p<0.001$). Expression of p75NTR peaks post-symptomatically in SOD1G93A mice between P100-120 days, but represented less that 5% of the total ChAT +ve population. Additionally, those motor neurons expressing p75NTR were significantly larger than those not expressing the receptor ($p<0.01$) and there was minimal co-expression with ATF-3, a marker for cell stress/pre-apoptosis. Finally, we confirmed that MLR2-488 was transported to motor neuron cell bodies in the spinal cord after intraperitoneal injections in symptomatic SOD1G93A mice (n=4). Analysis of these findings suggest that p75NTR is expressed by affected motor neurons for only a short time period suggesting that any therapy specifically targeting these neurons with p75NTR antibodies will require frequent injections or sustained administration beginning well before the onset of symptoms. (238 words)

POS-THU-157

ENHANCEMENT OF REACHING ABILITY AND REDUCED CORTICAL ATROPHY FOLLOWING TRANSPLANTATION OF NEURAL PROGENITORS GENERATED FROM HUMAN EMBRYONIC STEM CELLS IN A RAT MODEL OF CORTICAL ISCHEMIA.

Fahad Somaa, Jon Niclis, Mirella Dottori, Clare Parish and Lachlan Thompson.

Abstract

PURPOSE OF STUDY:

Here, we investigate whether transplanted neural progenitors generated from human embryonic stem (ES) cells can alleviate deficits in fine motor function in athymic (nude) rats that have received focal ischemic lesion of the frontal cortex through local infusion of endothelin-1 (ET-1). We also aimed to assess the capacity of grafts neurons to survive and integrate into a pathological environment.

RESULTS:

Intra-cortical grafting of human neural progenitors promotes significant recovery of forepaw use in rats with ischemic cortical region damage. This was seen as early as 4 weeks after grafting and was sustained up to 6 months. Histological examination of the grafted animals showed extensive integration of grafted neurons. Interestingly, a comparison of grafted animals with un-grafted controls showed that the grafts significantly prevented secondary cortical atrophy following ischemia.

Conclusion:

These results suggest that transplantation of neural progenitors may be a useful therapeutic strategy in treating certain types of brain damage. The early therapeutic benefit and the reduction of cortical atrophy suggest the primary mechanism of action is neuroprotective rather then cellular replacement. Nonetheless, the extensive capacity of transplanted neurons to integrate within the host brain suggests that at longer time-points cellular replacement may well add further benefit.

POS-THU-158

CELLULAR PATHOGENIC MECHANISMS INDUCED BY FUSED IN SARCOMA IN AMYOTROPHIC LATERAL SCLEROSIS

Sultana J[1], Atkin, J[1], Farg M[1], Al Helwani N[1], Billa N[1] and Walker A[1]

[1] Department of Biochemistry, La trobe Institute for Molecular Science, Melbourne

Amyotrophic Lateral Sclerosis (ALS) is a fatal adult onset neurodegenerative disease, causing the progressive degeneration of motor neurons. Mutations in the gene encoding fused in sarcoma (FUS) are responsible for causing a subset of ALS cases. In neurons, FUS expression is predominantly nuclear however, mutations lead to its cytoplasmic translocation followed by inclusion formation. Both oxidative and ER stress are implicated early in ALS pathology and the interplay between them is poorly understood. However both processes cause mutant FUS to co-localise within stress granules, which are thought to be early precursors to inclusions. In response to ER stress protein disulphide isomerase (PDI) is upregulated, and shown to be protective against FUS ALS pathology. However it is unknown whether this protective effect is a result of the chaperone or disulphide interchange activity of PDI. This study investigated the cellular pathogenic mechanisms induced by FUS and examined whether the chaperone or disulphide interchange activity was responsible for the protective effect of PDI. This study shows that induction of either oxidative or ER stress causes mutant FUS to recruit to stress granules and translocate to the cytoplasm. Also, induction of oxidative stress was found to induce ER stress. Both the chaperone and disulphide interchange activity of PDI decreased mutant FUS cytoplasmic translocation and reduced in ER stress, demonstrating that both activities of PDI were protective against ALS FUS pathology. These findings further support the notion that interplay between oxidative and ER stress exists in ALS and raises the question of whether abnormal disulphide bonding occurs in FUS.

POS-THU-159

OPTINEURIN AND MYOSIN VI ASSOCIATED CELLULAR TRAFFICKING DEFECTS IN AMYOTROPHIC LATERAL SCLEROSIS.

Vinod Sundaramoorthy[1], Adam K Walker[1,2], Vanessa Tan[3], Manal A Farg[1], Gilles Guillemin[3], Julie D Atkin[1].

1. Department of Biochemistry, La Trobe University, Victoria, Australia. 2. Center for Neurodegenerative Disease Research, School of Medicine, University of Pennsylvania, Philadelphia, USA. 3. The Australian School of Advanced Medicine, Macquarie University, NSW, Australia 2109

Short range transport along actin filaments is essential for efficient trafficking of vesicular protein cargos and organelles within cell. Myosin family of motor proteins including myosin VI drives trafficking along actin filaments. Optineurin interacts with myosin VI connecting it to secretory vesicles and autophagosomes. Recently mutations in optineurin have been identified to cause inherited familial forms of Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder causing motor neuron death. In this study we identify that ALS mutations cause dissociation of optineurin from vesicular myosin VI motor complex, leading to diffused cytoplasmic distribution in NSC-34 cells. ALS mutant optineurin induces ER stress and Golgi fragmentation, parallel with inhibition of secretory protein trafficking from Golgi to plasma membrane. This study also provides new information on the function of optineurin. We show that optineurin in association with myosin VI is involved in trafficking and tethering of lysosomes aiding their fusion with autophagosomes. Expression of ALS mutant optineurin or knockdown of optineurin blocks the fusion of lysosomes with autophagosomes resulting in accumulation of autophagosomes. We further show that optineurin is diffused from vesicular structures in the motor neurons of sporadic ALS patient. Also we observed reduced binding of optineurin with myosin VI in sporadic ALS patient spinal cord lysates compared to non-neurological controls. These results indicate optineurin and myosin VI associated trafficking defects occurs in familial as well as sporadic forms of ALS explaining important pathogenic features such as defective autophagy and ER-Golgi dysfunction observed in both forms of ALS. (246 words).

POS-THU-160

MOTOR END PLATE (MEP) TARGETING INCREASES UPTAKE AND ADENOVIRAL TRANSDUCTION IN SPINAL CORD MOTOR NEURONS, DORSAL ROOT GANGLIA (DRG) AND MYOFIBRES

Andrew Paul Tosolini, Rahul Mohan and Renée Morris

Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, NSW 2052, Australia

Viral-mediated gene therapy can take advantage of the relationship between a muscle and its innervating motor neurons to deliver therapeutic genes. In a gene therapy scenario, intramuscular injections and the subsequent retrograde delivery of the viral vector is a minimally invasive way to transduce myofibres as well as the innervating sensory and motor neurons. We have previously shown that targeting the entire length of muscles' MEP regions significantly increases the uptake of a retrograde tracer into corresponding motor neurons (*1*). The aim of this study was to determine if targeting the entire MEP region of a muscle with adenovirus would significantly increase expression of the transgene within mouse spinal cord motor and sensory neurons. Recombinant adenovirus serotype 5, driven by the CMV promoter and encoding the reporter tag-GFP (Ad-GFP) was injected along various points of the MEP region of the triceps brachii muscle. Mice were subsequently intra-cardially perfused and their spinal cord, ventral and dorsal roots, DRG and targeted skeletal muscle were dissected out, sectioned and the tissue was analysed under epifluorescence to see the presence of GFP. This analysis showed that targeting the MEPs with Ad-GFP produced significant expression of GFP within spinal cord motor neurons. Moreover, GFP expression was also present within the ventral and dorsal roots, DRG and triceps brachii myofibres. This study suggests that targeting muscles' MEP regions with an adenovirus is an effective and minimally invasive way to retrogradely deliver therapeutic transgenes into spinal cord motor neurons, DRG and myofibres.

1) Tosolini et al., (2013) Front. Neurol. 4:58.

(245 words)

POS-THU-161

NEURAL MECHANISMS OF PERISTALSIS IN THE ISOLATED RABBIT COLON: A NEUROMECHANICAL LOOP HYPOTHESIS.

Wiklendt L[1], Dinning PG[1,2], Spencer NJ[1], Brookes SJH[1], Costa MC[1].

1. Human Physiology and Centre for Neuroscience, FMST, School of Medicine, Flinders University. 2. Department of Gastroenterology & Surgery. Flinders Medical Centre. Bedford Park, SA 5042

Intestinal propulsion is mostly due to the aboral propagation of rings of circular muscle contractions (peristaltic contractions) that are controlled by enteric neural circuits. However, evidence that they operate during actual peristaltic contractions is still lacking. We have used a newly developed method(1) to deduce the state of neural excitation and inhibition from the mechanical states of the intestinal muscle, to identify the role of enteric reflex pathways in the propagation of peristaltic contractions in the isolated rabbit colon. Methods: *Ex-vivo* segment of distal colon taken from six rabbits were kept distended by intraluminal Krebs solution to initiate irregular peristaltic contractions. Results: From the combined pressure/diameter spatiotemporal maps, 25.8 ± 18.2 propagating contractions/10min were recorded. These propagating contractions were associated with an advancing region of dilation and that this region of dilation was significantly greater immediately prior to propagating contractions, in comparison to the dilated regions associated with non-propagating contractions (2.7 ± 1.4mm v 1.6 ± 1.2mm; $P < 0.0001$). The increased dilatation, preceding the propagating contractions was significantly ($P < 0.005$) more likely to be associated with a preceding active relaxation compared to the non-propagating contractions. Conclusions: The propagation of the peristaltic contractions requires ongoing reactivation of ascending enteric excitatory reflex pathways by the advancing bolus that acts as distending stimulus. This neuro-mechanical loop hypothesis of peristaltic contractions is consistent with the original proposal by Bayliss and Starling (1899). (230 words)

1. Costa MC, et al. Front Syst Neurosci 2013, **7**:7.

POS-THU-163

UTILISING THE MULTIBAC BACULOVIRUS PROTEIN EXPRESSION SYSTEM TO EXPRESS THE Γ- SECRETASE ENZYME COMPLEX.

Imran KHAN[1,2,3], Sudarsan Krishanswamy[4], David Groth[3], Linda Wijaya[1], Mike Morrici[1], Paul Fraser[5], Imre Berger[6], Christiane Schaffitzel - Berger[6], Ralph Martins[1,2] and Giuseppe Verdile [1,2,3]

1. Centre of Excellence for Alzheimer's Disease Research & Care, School of Medical Sciences, Edith Cowan University, Joondalup WA 6027, Australia.
2. School of Psychiatry and Clinical Neurosciences, The University of Western Australia Crawley, WA 6009, Australia.
3. School of Biomedical Sciences, Curtin University, Bentley WA, 6102
4. Australian Neuromuscular Research Institute (ANRI), Australian Neuro-Muscular Research Institute, The University of Western Australia.
5. Centre for Research into Neurodegenerative Diseases (CRND), University of Toronto, Toronto, Canada
6. European Molecular Biology Laboratory, Grenoble, France

ABSTRACT

Baculoviral protein expression in insect cells has been traditionally used to generate large amounts of the protein of interest for biochemical or structural analysis. For multi-protein complexes, expression of several genes in one cell can be achieved by co-infection with several viruses, each carrying a single foreign gene. However, the logistical demand of maintaining viruses at known titers and establishing relative equal expression levels render large-scale multi-protein complex production very difficult. The Multi-bac baculovirus protein expression system has made it possible to infect with one single virus containing all components of the complex of interest, resulting in a larger protein yield. Using this system, we aimed to reconstruct the γ-secretase complex, an enzyme essential in the production of beta amyloid, a key protein in neurodegenerative process in Alzheimer's disease.

A MultiBac vector containing all components of the γ-secretase complex was generated and successful expression was observed for all components. The complex was shown to be active in processing the direct precursor to Aβ, APP-C99 and interactions between all components were observed by co-immunoprecipitation studies. In addition we have included protein purification tags on two of the component proteins to facilitate two-step affinity purification.

We have utilized the Multi Bac protein expression system to successfully generate an active γ-secretase complex. This now allows larger scale production and purification of the complex for further analysis. In conjunction with available automated multi-complex generation, this expression system also allows the opportunity to generate permutated γ-secretase variants with modified or mutated subunits in high throughput.

POS-THU-164

TRANSCRANIAL MAGNETIC STIMULATION OF HUMAN DENTAL PULP STEM CELLS IN THE MAMMALIAN BRAIN

Kremer, KL[1,2,4], Smith, AE[1], Sandeman, L[1,2], Inglis, J[1,3,4], Ridding, MC*[1] and Koblar, SA*[1,2,4]
*Joint senior authors

[1]The Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia [2] School of Medicine, The University of Adelaide, Adelaide, South Australia [3]Centre for Stem Cell Research, The Robinson Institute, University of Adelaide, Adelaide, South Australia [4]Stroke Research Programme, School of Medicine, The University of Adelaide, Adelaide, SA 5005, Australia.

The burden of stroke on the community is growing, and therefore, so is the need for a therapy to overcome disability following stroke. Cellular-based therapies are being actively investigated at a pre-clinical and clinical level. Studies have reported the beneficial effects of exogenous stem cell implantation, however, the benefits reported are associated with the survival of a small number of implanted stem cells. This study investigated the use of a complementary therapy of transcranial magnetic stimulation (TMS) following implantation of human dental pulp stem cells (huDPSC) in the rodent cortex. Sprague-Dawley rats were anaesthetised and injected with $6x10^5$ huDPSC or control media via an intracranial injection, and then received real TMS ($TMS_{0.2Hz}$) or sham TMS (TMS_{sham}) every 2nd day for 2 weeks. Brain sections were analysed for the survival, migration and differentiation characteristics of the implanted huDPSC. In animals treated with huDPSC and $TMS_{0.2Hz}$ there were significantly less implanted huDPSC and those that survived remained in the original cerebral hemisphere compared to animals that received TMS_{sham}. We suggest that TMS may cause an increase in glutamate levels, which promotes an unfavourable environment for stem cell implantation, proliferation and differentiation. It should be noted that only one paradigm of TMS was tested as this was conducted as a pilot study, and further TMS paradigms should be investigated in the future. (215 words)

POS-THU-165

DEVELOPMENT OF A NOVEL ANTEROGRADE TRACING TECHNIQUE TO SELECTIVELY IDENTIFY THE DIFFERENT TYPES OF SPINAL AFFERENT NERVE ENDING THAT UNDERLIE NOCICEPTION FROM VISCERAL ORGANS

M.Kyloh[1] & N.J. Spencer[1]

1. Discipline of Human Physiology, School of Medicine, Flinders University, SA 5042, Australia

Background: One major weakness in our understanding of pain perception from visceral organs is our lack of knowledge in the location, morphology and neurochemistry of all the different types of spinal afferent nerve endings in visceral organs, that detection of noxious and innocuous stimuli. This is because we lack techniques to selectively label only spinal afferents. Our aim was to develop an anterograde tracing technique that labels only spinal afferent nerve endings in visceral organs, without also labelling all other classes of extrinsic afferent and efferent nerves. Methods: Mice were anesthetized with isoflurane and dextran-biotin injected, *via* glass microelectrodes, into L6 and S1 dorsal root ganglia (DRG). Mice recovered for 7 days, were then euthanized and the colon removed. Results: In 14 mice, anterograde labelling revealed 11 unique classes of afferent endings that terminated within multiple distinct anatomical layers of the colon and rectum, a substantial proportion of which were immunoreactive to the sensory peptide, CGRP. Three major types of endings were identified in the circular muscle, which had maximum projections of 827 ± 227µm around the circumferential axis of the colon and 111 ± 14µm in the rostral to caudal (oral to anal) axis of the colon (N=4). Conclusions: We demonstrate a new technique that facilitates selective anterograde labelling of spinal afferent nerve endings in visceral organs. We have identified the different classes of spinal afferent nerve ending that innervate the large intestine and reveal a level of complexity of sensory processing far greater than we ever expected.

POS-THU-166

TARGETED NANOPARTICLE BASED THERAPIES TO TREAT SECONDARY DEGENERATION FOLLOWING PARTIAL INJURY TO THE CNS

Lozić I[1,2], Bartlett CA[1], Smith N[1,2], Kilburn MR[3], Iyer KS[2], Dunlop SA[1], and Fitzgerald M[1]
[1]Experimental and Regenerative Neurosciences, School of Animal Biology; [2] School of Chemistry and Biochemistry; [3] Centre for Microscopy, Characterisation and Analysis, UWA, 35 Stirling Hwy, Crawley WA 6009

Oxidative stress in the astrocytic syncytium adjacent to the primary injury is believed to play a vital role in the spread of secondary degeneration following injury to the CNS. In order to stem the spread of secondary degeneration, we have developed a targeted nanoparticle-based therapy designed to deliver the anti-oxidant resveratrol to astrocytes overexpressing aquaporin 4 (AQP4) following neurotrauma. Nanoparticles (NPs) containing 6 nm magnetite particles, Rhodamine B dye and resveratrol were synthesised using a spontaneous emulsion method from a highly functionalised poly(glycidyl methacrylate) polymer [141 nm]. A custom-made anti-AQP4 antibody was attached to the nanoparticles via a melemide-thiol reaction [NP size: 581 nm]. *In vitro*, antibody conjugated resveratrol encapsulating NPs appeared to be taken up by a larger proportion of mixed retinal cells than non-antibody conjugated resveratrol encapsulating NPs, or antibody conjugated NPs without resveratrol [antibody conjugated resveratrol encapsulating NPs = 0.31 ± 0.05; non-antibody conjugated resveratrol encapsulating NPs = 0.19 ± 0.03; antibody conjugated NPs without resveratrol = 0.20 ± 0.03, data expressed as the proportion of cells with NP uptake]. PVG rats that underwent partial optic nerve transection and had antibody conjugated resveratrol encapsulating NPs nano-injected into the injury site at the time of injury, made a greater number of responses in the optokinetic nystagmus visual reflex test, than similarly injured animals treated with non-antibody conjugated resveratrol encapsulating NPs ($P \leq 0.05$). NPs containing resveratrol targeted to astrocytes may serve as an effective therapy for secondary degeneration following neurotrauma.

POS-THU-167

BEHAVIOURAL STATE CONTROL BY NUCLEUS INCERTUS (NI) NETWORKS: STUDIES USING AAV-DELIVERED DESIGNER RECEPTORS (DREADDs)

Ong-Pålsson EKE[1,2], Hawkes D[1], McDougall SJ[1,2], White MD[1,5], Bathgate RAD[1-3], Ma S[1,2], Gundlach AL[1,2]

[1]The Florey Institute of Neuroscience and Mental Health, [2]Florey Department of Neuroscience and Mental Health [3]Departments of [3]Biochemistry and Molecular Biology, [4]Anatomy and Neuroscience, The University of Melbourne, Victoria, Australia; [5]ARMI, Monash University, Victoria, Australia

Anatomical and physiological studies suggest the *nucleus incertus* (NI) plays a key role in behavioural state control via interactions with brainstem and forebrain circuits (Ryan PJ *et al* . 2011). Our research aims to determine how NI networks influence brain activity patterns in limbic and septohippocampal pathways, and related physiology and behaviour in different contexts. In these experiments, adult (Sprague Dawley) rats (n=6) received bilateral injections into NI of an AAV-excitatory (hM3Dq) 'Designer Receptor Exclusively Activated by Designer Drug' ('DREADD')-mCherry vector (2×10^8 genomic copies/µl). After 2-5 wk, we examined the consequences of hM3Dq-DREADD activation by the inert ligand, clozapine-N-oxide (CNO; 3 mg/kg, i.p.). All neurochemical, physiological and behavioural parameters were assessed relative to CNO treatment of rats injected with an AAV-mCherry control (n=4), or saline treatment. Relative to control, CNO activation of hM3q in NI produced: (*i*) increased Fos-IR in NI neurons; (*ii*) depolarisation of transduced NI cells in an *in vitro* brain slice; (*iii*) marked, long-lasting alterations in home cage EEG activity (n=6); (iv) an increase in homecage feeding behaviour (n=4, $P<0.01$); and (*v*) disrupted spatial memory in the Y-maze (n=4, $P<0.05$). Our data, together with previous findings, further indicate a strong modulatory role of NI networks in the control of behavioural state and complex behaviour. Future studies will explore the impact of hM4Di-DREADD-mediated *inhibition* of NI neurons and begin to dissect the functional topography of specific NI projections (e.g. relaxin-3 positive/negative) in relation to normal physiology and disease-related pathology.

(241 words).

POS-THU-168

ELECTRICAL FIELD STIMULATION (EFS) OVER SACRAL NERVES OVERCOMES CHRONIC CONSTIPATION DUE TO ANORECTAL RETENTION (AR) IN CHILDREN – A PILOT STUDY

YI Yik,[1,2,3,4] JM Hutson,[1,2,3] BR Southwell[1,3] –

1. Murdoch Childrens Research Institute, Parkville, Australia,
2. Dept. Urology, Royal Childrens hospital, Parkville, Australia,
3. Dept. Paediatrics University of Melbourne, Parkville, Australia,
4. Dept General Surgery, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Purpose: When the bowel functions normally, stool does not accumulate in the rectum. In the most common form of chronic constipation, stools accumulate in the rectum (anorectal retention, AR) causing dilation of the rectum with loss of sensory nerve activation. In organ bath physiology, nerves controlling the bowel respond to electrical field stimulation (EFS) by altering bowel contractions. Can this translate into a therapy? The physiotherapy method of transcutaneous electrical stimulation (TES) is a form of EFS. Aim: Pilot study to test the effect of TES on children with AR. Method: Children with chronic constipation resistant to laxative treatment had radionuclear gastrointestinal transit studies (scintigraphy) confirming AR. Parents/children were trained to administer home-based TES using battery-powered interferential stimulator with 4 pad electrodes (4cmx4cm), 2 on back over sacral nerves and 2 on front at same level, connected so currents crossed. Stimulation 80-150 Hz beat, 4 kHz carrier frequency, 20-30 mAmp. Results: Nine children (5-10yrs, mean: 8yrs) administered home-based TES for 1-hour/day for 3 months. Bowel function changed with an increase in defecation from (mean±SEM) 0.8±0.5 bowel actions/wk (pre) to 4.4±1.6 bowel actions/wk (post, p=0.03). Soiling reduced from 6.0±1.9 days/wk to 1.4±1.1 days/wk, p=0.0001. Abdominal pain reduced (2.2±0.5 days/wk to 0.4±0.5 days/wk, p<0.001). 6 children developed sensory awareness of rectal filling suggesting altered neural responses. Conclusion: Electrical field stimulation delivered as TES altered rectal sensory awareness and changed defecation in children with chronic constipation associated with stool accumulating in the anorectum. EFS could translate into an effective treatment for chronic constipation. (250)

POS-THU-169

ORGANELLE TRANSPORT IS DISRUPTED IN HEREDITARY SPASTIC PARAPLEGIA PATIENT STEM CELLS

Wali G1, Fan Y1, Sutharsan R1, Sue C2, Crane D, Fernandes J1 and Mackay-Sim A1

1. National Centre For Adult Stem Cell Research, Eskitis Institute for Drug Discovery, Griffith University, Brisbane, QLD. 2. Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St Leonards, NSW.

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder that leads to progressive gait disturbances with lower limb muscle weakness and spasticity. Mutations in *SPAST* gene are a major cause of adult-onset, autosomal-dominant HSP. Spastin, the protein encoded by *SPAST*, is a microtubule-severing protein that is enriched in the distal axon of corticospinal motor neurons, which degenerate in HSP patients. Patient-derived olfactory stem cells (ONS cells) from *SPAST* HSP patients have significant disease-associated dysregulation of microtubule-associated genes, compared to ONS cells from healthy controls. Patient-derived ONS cells have significantly reduced expression of acetylated alpha-tubulin, observed in Western blots and in quantitative analysis of cell images, indicating a reduction in stabilised microtubules, and significantly altered intracellular distributions of two organelles, peroxisomes and mitochondria. The dynamics of intracellular trafficking of peroxisomes and lysosomes was visualised by fluorescent labelling and time-lapse imaging in living cells. Organelle trafficking was quantified using automated image analysis to follow at least 300 peroxisomes or lysosomes in each of 10 patient-derived and 10 control-derived cell lines, both as undifferentiated ONS cells and after differentiation into neuron-like cells with long neurites. The results show that peroxisome transport is significantly slower in patient-derived cells compared to control-derived cells. Paradoxically, lysome transport is significantly faster in patient-derived cells. These patient-control differences were greater in differentiated axon-like processes that contained microtubules but not actin, suggesting an explanation for the neural phenotype of this disease. This is the first evidence for trafficking deficits in HSP patient-derived cells. (244 words)

POS-THU-170

HUMAN DENTAL PULP STEM CELLS MODULATE PERMEABILITY OF THE BLOOD-BRAIN BARRIER.

Winderlich JN, Kremer K, Koblar SA.
Stroke Research Programme, Discipline of Medicine, School of Medicine, University of Adelaide.

Intravenously administered stem cells transmigrate the blood-brain barrier (BBB) via an unknown mechanism. The objectives of this study were to develop and characterise a reproducible and physiologically accurate model of the BBB. This *in vitro* BBB model was used to demonstrate the ability of human adult dental pulp stem cells (DPSC) to modulate BBB permeability and to analyse the expression of known permeability factors by stem cells. An *in vitro* model of the BBB was developed by co-culturing human bone marrow endothelial cells (BMEC) and rat astrocytes on opposite sides of a porous membrane. This model was used to show that soluble factors expressed by DPSC were capable of increasing BBB permeability. SDF-1 treated DPSC showed a dose-dependent increase in their ability to cause permeability. VEGF-a has been implicated as a candidate permeability factor and we found that exposure of the *in vitro* BBB to a VEGF-a receptor antagonist attenuated the ability of DPSC conditioned media to cause permeability. This study demonstrated that the *in vitro* BBB model can be used to assay biologically active substances with respect to BBB permeability. DPSC were shown to be capable of mediating an increase in BBB permeability via the expression of VEGFa. (228 words).

POS-THU-171

MICROFLUIDIC CULTURE PLATFORM FOR STUDYING NEURONAL RESPONSE TO AXONAL INJURY.

Yap YC[1], King AE[2], Breadmore MC[3], Guijt RM[4], Dickson TC[1].

1. Menzies Research Institute, Tasmania, Australia. 2. School of Medicine, University of Tasmania, Australia. 3. School of Chemistry, University of Tasmania, Australia. 4. School of Pharmacy, University of Tasmania, Australia.

We have developed a new model of localised axonal stretch injury by combining a thin, flexible poly(dimethylsiloxane) (PDMS) membrane with microfluidic isolation of neuronal soma and axons. The membrane will deflect upward and stretch the axons when pressurizing a microfabricated air channel beneath the membrane. A very mild (0.5 % strain) or mild stretch injury (5 % strain) was applied to cortical neurons after 7 days *in vitro* (DIV). To quantify the extent of distal axonal degeneration, we calculated the degenerative index (DI) of tau immunolabelled images of fixed axons at 24 h and 72 h post injury (PI) time points. A degeneration index (DI) was calculated as the ratio of fragmented axon area over total axon area. Under very mild injuries (0.5 %), the majority of the axons remained intact and healthy at 24 h PI with no significant difference in DI when compared to the control. However, the degenerative index increased significantly at 72 h following the very mild injury (DI=0.11±0.03). When the injury level was increased (5 % injury,
mild injury), we observed microtubule fragmentation as well as the formation of numerous axonal swellings along the injured axons at both time point, with a significant increase in DI at 24 h PI (DI=0.17±0.02) and 72 h PI (DI=0.18±0.01). These responses are similar to neuronal response to trauma that observed *in vivo*, suggesting this model can be used to study stretch injury and will provide additional insights when testing with different potential therapeutic agents. (246 words)

POS-THU-172

ARE SHARKS “SMART”? DEVELOPING QUANTITATIVE MEASURES OF COGNITIVE ABILITY IN FISHES

Yopak KE[1], Goh G[1], Heel K[2], Wassell R[1], Northcutt RG[3], Collin SP[1]

1. School of Animal Biology and the UWA Oceans Institute, University of Western Australia, Crawley, WA 6009, Australia. 2. Centre for Microscopy, Characterisation, and Analysis, University of Western Australia, Crawley, WA 6009, Australia. 3. Department of Neurosciences and Scripps Institution of Oceanography, University of California San Diego, USA.

Relationships between the body, brain, and major brain regions have traditionally been used to infer cognitive abilities across all vertebrates, providing vital information about life history traits, behavior and “intelligence”. Sharks and their relatives were the first group to exhibit the highly homologous vertebrate brain archetype, which includes the olfactory bulbs, telencephalon, diencephalon, mesencephalon, cerebellum, and medulla. Indeed, we show that broad variability in the size and complexity of these brain areas is highly correlated with habitat and/or specific behavior patterns in sharks. However, a new method of accurately assessing the number of neurons in the brain in mammals, isotropic fractionation, suggest that brain mass may be a poor predictor of cognitive ability and enhanced associative function. Here, we present the first application of this technique in fishes, using the Port Jackson shark, *Heterodontus portusjacksoni*. The total number of neurons (as compared to non-neuronal glia) was measured using the isotropic fractionation method. Counts made on cell nuclei were automated via flow cytometry and concordance between automated and manual counting methods was further tested using a haemocytometer. Here we detail the strategies for the optimisation of brain homogenisation, neuronal staining, calibration, imaging, and quantification of brain cells in fishes. Our results show a significant similarity in neuronal scaling across vertebrates and we suggest the isotropic fractionation method may serve as an effective tool to assess functional ability and processing power in the brains of fishes, with implications for how “intelligence” has evolved across vertebrates. [243 words]
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POS-THU-173

MORPHOLOGY AND CONNECTIVITY OF A BISTRATIFIED AMACRINE CELL IN THE MOUSE RETINA

S.C.S. Lee[1,2], A. Meyer[3], K. Dedek[3], and S. Haverkamp[1]

[1]Max Planck Institute for Brain Research, Frankfurt, Germany.

[2]University of Sydney - Save Sight Institute, Sydney, NSW 2000, Australia.

[3]University of Oldenburg, Oldenburg, Germany.

Purpose: There are at least 20 different types of amacrine cells in the mammalian retina and here we describe the morphology, connectivity, and the development of a glycinergic amacrine cell, the A8 amacrine cell, in the mouse retina.

Methods: A8 amacrine cells were identified in a transgenic mouse line where green fluorescent protein is expressed under the *thy1* promoter. Retinas were triple labeled with bipolar and synaptic markers to determine the synaptic connectivity of A8 cells. Antibodies to C-terminal binding protein (CtBP2) were used to identify ribbon synapses; antibodies against glycinergic receptors were used to identity potential postsynaptic partners. In addition, we injected single A8 cells with Neurobiotin to determine cell coupling.

Results: We found an average of 276±65 CtBP2 puncta per cell for the ON plexus and 367±84 puncta for the OFF plexus (n=10 cells). 49% of the CtBP2 puncta in the OFF plexus are from t2 cone bipolar axons (n=7 cells). 39% of the CtBP2 puncta in the ON plexus are from t6 bipolar cell axons (n=4 cells) and only few are from rod bipolar axons (7%; n=4 cells). In addition, A8 cells are coupled to ON cone bipolar cells and provide putative inhibitory feedback *via* GlyRα1 to OFF cone bipolar cells. Furthermore, we found evidence that A8 cells provide glycinergic output *via* GlyRα1 onto sustained A-type ganglion cells.

Conclusion: We predict the A8 cell functions as an ON-OFF crossover-inhibiting cell with a role in modulating sustained responses of A-type ganglion cells.

(244/250 words)

POS-THU-174

KETAMINE AND ITS METABOLITE INHIBIT LIPOPOLYSACCHARIDE (LPS)-INDUCED INTERLEUKIN-6 PRODUCTION IN A TIME- AND CONCENTRATION-DEPENDENT MANNER: POTENTIAL INVOLVEMENT OF MULTIPLE PATHWAYS

Li Y[1], Hutchinson MR[2], Coller JK[1], Somogyi AA[1]

1. Discipline of Pharmacology & 2. Discipline of Physiology, School of Medical Sciences, The University of Adelaide, Adelaide, SA

Racemic ketamine inhibits post-operative interleukin-6 (IL-6) inflammatory response, possibly via the suppression of Toll-Like receptor 4 (TLR4) signalling. However, the contribution of each enantiomer and their corresponding active metabolites, *S*- and *R*-norketamine, to such anti-inflammatory activity is unclear. We examined the effect of ketamine and norketamine enantiomers on LPS-induced IL-6 production using HEK293 cells stably expressing human TLR4 and co-signalling molecules. LPS-induced IL-6 concentrations after pre-incubation with ketamine and norketamine enantiomers at different concentrations (1, 10, 100 μM) and exposure times (20 min to 8 h) were quantified using ELISA. The time-course response of ketamine on IL-6 production exposure was also examined by removing the supernatants at the end of pre-incubation prior to LPS stimulation. The inhibitory effects of ketamine and norketamine on induced IL-6 production were concentration-dependent (100 > 10 > 1 μM). Inhibitory effects of S-ketamine and S-norketamine on IL-6 production were enhanced by longer pre-incubation times whereas the effect of R-ketamine on IL-6 production was decreased. IL-6 production was inhibited by both ketamine enantiomers when drug was removed from the supernatant after 4 h pre-incubation ($p < 0.0001$) but not after 20 min pre-incubation ($p > 0.34$). The time course and stereoselective difference in inhibition of LPS-stimulated IL-6 production by ketamine and norketamine enantiomers may reflect a mechanistically-based difference between the acute and long-term anti-proinflammatory activity of ketamine. The mechanism of acute activity is likely associated with inhibition of TLR4 binding, whereas the long-term activity appears to be independent of TLR4 receptor binding. (246 words)

POS-THU-175

MOLECULAR CHARACTERIZATION OF MOUSE PARVALBUMIN POSITIVE PROPRIOCEPTIVE DORSAL ROOT GANGLIA NEURONS

Madden JF[1], Smith KM[1], Callister RJ[1], Jobling P[1] and Graham BA[1].

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle and Hunter Medical Research Institute (HMRI)

Dorsal root ganglia (DRG) contain the cell bodies of sensory neurons. These cells convey a range of sensory modalities into the central nervous system, including nociception, temperature, tactile sensations, and proprioception. The excitability of different DRG neuron populations, which is shaped by the expression of a range of voltage sensitive ion channels, is critical to their capacity to encode such stimuli. Therefore, in this study we have analyzed the expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel subunits in parvalbumin positive 'proprioceptive' DRG neurons. We used a transgenic mouse expressing green fluorescent protein (GFP) under a parvalbumin promoter, and compared HCN1-4 expression in parvalbumin positive and negative cells. DRGs were isolated from transgenic animals (n=4), dissociated in collagenase [10mg/ml] and plated. Targeted GFP cells and non-GFP controls were collected using a suction pipette and pooled for analysis (n=50 cells each). RNA was extracted from the 2 groups and subjected to quantitative real time PCR analysis using primers for GFP, HCN1-4 and β actin. Delta cycle threshold scores were calculated for GFP and HCN1-4 using the β actin data and compared using paired t-tests. These comparisons confirmed our isolation method with GFP expression enriched in the GFP sample and practically absent in controls ($p < 0.001$). HCN1 expression was similar in GFP and control samples, but HCN2-4 expression was significantly greater in the GFP sample ($p<0.05$). Together, these results suggest a more prominent role for HCN channels, and the I_h current they mediate, in shaping the excitability of proprioceptive DRGs.

POS-THU-176

ENCODING OF SIMPLE AND COMPLEX VIBRATORY STIMULI BY TACTILE MECHANORECEPTORS IN THE HUMAN FINGER PAD.

David A Mahns[1], Ingvars Birznieks[2,3], Vaughan Macefield[1,3].

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

Purpose: Numerous studies describe the capacity of human subjects to discriminate the intensity and frequency parameters of vibratory stimuli. However, few studies have provided a detailed analysis of the neural responses to sinusoidal vibration in human subjects. We undertook a neurophysiological investigation of the responses of low-threshold mechanorceptors in the finger pad to pure sinusoidal vibration and complex (Beat) vibratory stimuli.

Methods: 56 Unitary tactile afferents were recorded via tungsten microelectrodes inserted into the median nerve. Pure sinusoidal vibration (4,8,16,32,64,128 and 256Hz at 10-300µm) was applied to the centre of the unit's receptive field. Beat stimuli were composed of two sine waves [S1,S2] to achieve beat frequencies of 4,8,16,32,64 and 128Hz.

Results: At any given frequency individual units increased afferent discharge as stimulus amplitude increased, with a parallel increase in the proportion of inter-spike-intervals matching the periodicity of the sine wave, i.e. spikes were not randomly distributed but were entrained to the stimulus waveform. U shaped tuning (threshold) curves demonstrated that FAI units were most sensitive at 32-64Hz, whereas FAII units were most sensitive at 64-128Hz. SAI fibres displayed a similar sensitivity to FAI fibres with comparable entrainment of afferent spike activity. Entrainment of spontaneous activity in SAII units was observed at frequencies of 16-128 Hz. Responses to complex stimuli were modulated by the Beat [S1-S2] and entrained by the fine [(S1-S2)/2] frequency.

Conclusions: The present data suggest that the comparable entrainment of impulse activity may be distributed across all four major afferent classes for simple and complex tactile stimuli. (250 words)

POS-THU-177

NUTRIENT SENSING IN ENTEROCHROMAFFIN CELLS

Martin AM[1], Peiris H[1], Raghupathi R[1], Sutherland K[2], Young RL[3] and Keating DJ[1]

[1]Molecular and Cellular Neuroscience, Flinders University, Adelaide, [2]Centre for Sleep Health and Research, Department of Respiratory Medicine, Royal North Shore Hospital, Sydney,[3]Nerve-Gut Research Laboratory, Discipline of Medicine, The University of Adelaide.

Enterochromaffin (EC) cells in the gut mucosa provide the most significant source (90-95%) of total body serotonin (5-hydroxytryptamine, 5-HT) and may be important nutrient sensors. Ingestion of nutrients such as glucose and fatty acids increases the release of 5-HT from EC cells, consequently affecting gastrointestinal (GI) functions including motility and gastric emptying. In contrast to this, fasting increases the expression of the rate limiting enzyme for 5-HT synthesis in EC cells, Tph1. Despite this knowledge, the mechanisms which regulate these responses of EC cells to nutrient intake and fasting are not known. An EC cell isolation method pioneered in our laboratory has allowed us to isolate EC cells and study their nutrient sensing mechanisms. We find that fasting significantly increases Tph1 expression and that specific nutrients may contribute to this adaptive response. Single cell amperometry studies demonstrate that EC cells respond acutely to high external glucose to trigger 5-HT release. We have also compared the gene expression of different nutrient sensing proteins in EC cells from the colon and duodenum of mice. We observe significantly higher expression of the Na+-glucose transporter, SGLT1, and the fatty acid receptors FFAR4/GPR120 and GPR119 in colonic compared to duodenal EC cells. Preliminary findings suggest that some of these receptors are upregulated in mice exposed to a high fat diet. By understanding the nutrient sensing mechanisms present in EC cells, we gain insight into how different nutrients may influence GI functions through EC cell 5-HT release. (241 words)

POS-THU-178

A STOCHASTIC MODEL OF SHORT TERM PLASTICITY AND CALCIUM ION CHANNEL NOISE AT INNER HAIR CELL RIBBON SYNAPSES.

Moezzi B[1], McDonnell MD[1]

Computational and Theoretical Neuroscience Laboratory, Institute for Telecommunications Research, University of South Australia, Mawson Lakes SA 5095, Australia.

We propose two modifications to Sumner et al.'s (2002) stochastic vesicle release model of AN-IHC complex with the aim of producing simulated inter-spike-interval (ISI) statistics in AN fibres that provide an improved match with empirical data.

First, we introduced short term plasticity to the pre-synaptic release probability, in a manner analogous to standard stochastic models of cortical short term synaptic depression. This modification results in a similar distribution of vesicle release intervals (VRI) to that estimated from empirical data by Heil et al. (2007). Heil et al. showed AN ISI distributions were better described if the VRI distribution was assumed to be a mixture of an exponential function and a gamma function with shape factor 2, both having the same scale parameter. Our modification to the biophysical model of Sumner et al. results in simulated ISIs that match Heil et al.'s data.

Second, the model of Sumner et al. assumes the calcium concentration dependence of the release probability is due to voltage dependent calcium channels. Random fluctuations in the number of open and closed channels can be expected to cause variability in the vesicle release probability. We therefore additionally modified the model of Sumner et al. to include a stochastic model of calcium channel opening and closing.

Heil P et al. (2007) "Spontaneous activity of auditory-nerve fibres: insights into stochastic processes at ribbon synapses," *J. Neurosci.*, vol. 27, no. 31, pp. 8457-8474

Sumner C.J et al. (2002) "A revised model of the inner-hair cell and auditory-nerve complex," *J. Acoust. Soc. Am.*, vol. 111, pp. 2178

(247 words)

POS-THU-179

CONTRIBUTION OF C-TACTILE FIBRES TO INNOCUOUS MECHANORECEPTION: AN EXAMPLE OF DISTRIBUTED PROCESSING OR REDUNDANCY?

Nagi SS[1], Dunn J[2], Birznieks I[2,3], Mahns DA[1]

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

A myriad of studies have argued that tactile sensibility is underpinned exclusively by large-diameter mechanoreceptors. Intriguingly, their slow-conducting counterparts, termed C-tactile fibres (CTs), have gained little traction over the years with their functional significance remaining largely unexplored. Intriguingly, the capacity to detect weak monofilaments was reportedly preserved in the hairy skin (alone) of *two* patients with large-fibre dysfunction (Cole et al. 2006). In this project, we explored whether the role of CTs in tactile sensibility is limited to conditions wherein large fibres are absent, or whether CTs contribute to the perceptual construct of touch even in normal conditions. In 15 healthy subjects, monofilament detection thresholds were measured in the ulnar territory of hairy (dorsal hand) and glabrous (little finger and palm) skin prior to and following the blockade of myelinated and unmyelinated fibres. Myelinated fibre blockade was confirmed by the abolition of focal vibration, innocuous cold and sharp pain sensations. Conversely, unmyelinated fibre blockade abolished warm sensibility, whereas cold thresholds remained unchanged. We found that, although the tactile thresholds were somewhat elevated in the absence of myelinated fibres, the capacity of subjects to detect monofilaments (as-low-as ~1.6mN) remained intact. This effect was observed in both hairy and glabrous skin, which confirms our recent findings on the ubiquity of the CT-fibre network (Nagi and Mahns 2013). Interestingly, the blockade of C fibres alone resulted in elevated tactile thresholds. These observations suggest that CTs need not be regarded as an otherwise redundant tactile system, but appear to contribute to normal tactile activity. (250 words)
250 words)

POS-THU-180

SILDENAFIL AFFECTS RETINAL FUNCTION OF ANIMAL MODELS OF RETINAL DEGENERATION

Nivison-Smith L[1], Zhu Y[1], Whatham A[1,2], Bui B[3], Fletcher EL[4], Kalloniatis M[1,2,4]

[1]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia.
[2]Centre for Eye Health, University of New South Wales, Sydney, New South Wales, Australia.
[3]Department of Optometry and Vision Science, University of Melbourne, Parkville, Victoria, Australia.
[4]Department of Anatomy and MeuroScience, University of Melbourne, Parkville, Victoria, Australia.

Sildenafil, the active ingredient in Viagra, is used to treat erectile dysfunction by inhibiting cGMP-specific phosphodiesterase type 5 (PDE5). Sildenafil also has a mild inhibitory effect on PDE6, an active enzyme present in retinal photoreceptors. Clinical studies show a single sildenafil dose only transiently depresses electroretinogram (ERG) responses in normal subjects. However, in patients with comprised retinal tissue, such as those with Retinitis Pigmentosa (RP), photoreceptors are under stress and sildenafil usage may push photoreceptors beyond the threshold of normal function and trigger degeneration. We first tested this theory using the P23H-3 rat, a rodent model for RP. We found that a-wave and b-wave responses were transiently decreased after a single sildenafil dose. However, after 6 consecutive daily doses, ERG responses were significantly reduced indicating that sildenafil could worsen inherited retinal degeneration. We then assessed the effects of sildenafil the heterozygous +/rd mouse, a model for heterozygous carriers of RP. Interestingly, *+/rd* mice demonstrated a significant increase in a-wave and b-wave amplitudes 1h after a single sildenafil dose. This was followed by a decrease in ERG response, two days and two weeks after the initial dose. ERG responses were dose dependent. Anatomically, only a slight increase in retinal cell death in +/rd mice was seen at the highest sildenafil dosage. However, cytochrome C, upstream trigger for apoptosis when released from mitochondria was increase in various retinal regions. These results have considerable implications as it is estimated that 1:50 humans are heterozygous carriers of inherited retinal dystrophy. (246 words)

POS-THU-181

MULTIPLE ACTIONS FOR VINPOCETINE FOLLOWING ACUTE RETINAL METABOLIC INSULT

Nivison-Smith L[1], Acosta ML [2,3],O'Brien BJ [2,4], Misra S [2,3,5], Ready S [2], Truong M [2], Guo CX [2], Kalloniatis M [1,2,3,6]

[1]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia.
[2]Department of Optometry and Vision Science, University of Auckland, Auckland, New Zealand.
[3]New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand.
[4]National Vision Research Institute, Carlton, Victoria, Australia.
[5]Department of Ophthalmology, University of Auckland, Auckland, New Zealand.
[6]Centre for Eye Health, University of New South Wales, Sydney, New South Wales, Australia.

Vinpocetine is a natural drug which exerts neuroprotective effects on the brain and retina through actions on glucose metabolism, cation channels, glutamate receptors and other pathways. We investigated the effect of vinpocetine in the retina after acute metabolic insult using *ex vivo* retinal cultures subjected to normoxia, ischemia, hypoxia and hypoglycemia and an *in vivo* model of ischemia-reperfusion. We found that vinpocetine increased glucose availability and metabolic activity (assessed by lactate dehydrogenase (LDH) activity) of the normal retina. Vinpocetine also modulated cation channel permeability of specific ganglion cell populations in the normal retina when assessed by the probe, 1-amino-4-guanidobutane (AGB). Following ischaemia or hypoglycaemia, retinae without vinpocetine showed elevated LDH activity and unregulated AGB entry into calretinin and parvalbumin containing inner retinal neurons. Vinpocetine reduced LDH activity and cation channel permeability of most amacrine and ganglion cell populations to normal levels. Vinpocetine also recovered calretinin and parvalbumin immunoreactivity lost during ischaemia suggesting neuroprotective mechanisms mediated by regulation of intracellular calcium. Vinpocetine exerted similar effects on metabolic activity and cation channel permeability for *ex vivo* retinae subjected to *N*-methyl-D-aspartate (NMDA) but not kainate activation suggesting vinpocetine's may partly involve NMDA sensitive glutamate receptors. Thus vinpocetine's effects during retinal ischaemia involves multiple actions includes regulation of cation channel permeability, altering glucose metabolism and modulating NDMA sensitive glutamate receptors (216 words).

POS-THU-182

NOVEL APPLICATION OF A LIQUID CRYSTAL DISPLAY TO MAP RECEPTIVE FIELDS REVEALS HIGH ACUITY IN LIGHT ADAPTED PHOTORECEPTORS OF THE AUSTRALIAN BLUE-BANDED BEE

Schuckel, J[1,2], Wiederman SD[3], Warrant, EJ[1], O'Carroll DC[3]

1. Department of Biology, University of Lund, Lund, Sweden. 2. Smithsonian Tropical Research Institute, Panama City, Panama. 3. Adelaide Centre for Neuroscience Research, The University of Adelaide, Adelaide, Australia

The Australian blue-banded bee (*Amegilla)* is a fast flyer that also hovers during flight much like some flies do. What characteristics of the eyes allow for their flight behavior? Recent advances in liquid crystal display (LCD) technology allow for high resolution, fast refresh rates (120 Hz) and relatively high luminance, approaching that of natural conditions. We applied such a screen to map receptive fields of photoreceptors in fine detail, using scanning black objects and bars. This provides an alternative to traditional methods for characterizing angular sensitivity, which typically use dark-adapted states and impulsive flashes from a point light source. □□□□□preliminary data confirm the usefulness of LCD displays, in combination with models for optical blur as a fast, reliable and accurate means to study angular resolution in insects. We recorded intracellularly from photoreceptors in the acute zone (frontal eye field) of *Amegilla murrayensis,* and several other comparison species of diurnal insects (honeybee *Apis mellifera* & and dragonfly, *Hemicordulia australiae*). *Amegilla* photoreceptors have a slightly elongated visual field vertically with acceptance angles among the smallest reported in insects ($\Delta\rho_{\square}$=1.23° horizontally and $\Delta\rho_{v}$=1.4° vertically, N=2) and almost half those obtained using the same method from the honeybee ($\Delta\rho_{\square}$=2.09°, $\Delta\rho_{v}$=2.49°, N=4). *Apis* data are in close agreement with both physiological and behavioral methods reported previously. *Hemicordulia* acceptance angles are considerably smaller than dark-adapted values reported previously for this species ($\Delta\rho_{\square}$=0.87°, $\Delta\rho_{v}$=1.07°, N=4). Our data suggest acuity in *Amegilla* among the highest yet observed in insects. (240 words)

POS-THU-183

TIMESCALES OF SENSORY INTEGRATION IN VISUAL CORTEX ZAVITZ E, HAGHGOOIE S,

Yu HH, Davies AJ, Rosa MGP, Price NSC Department of Physiology,
Monash University, Clayton, Victoria, Australia

Behavioural choices enforce a trade-off between speed and accuracy. For example, sensory information can be integrated for long periods of time, leading to more accurate choices at the expense of slow reaction times. Here, we explore the temporal integration of sensory information by single neurons.

We recorded visually-evoked neuronal activity from 96 electrode “Utah” arrays implanted in the middle temporal area of marmosets (*Callithrix jacchus)* anaesthetised with sufentanil (8 µg/kg/h) and N_2O (70% in oxygen). Visual stimuli were dot patterns that moved coherently in a single direction. Stimulus directions were chosen randomly from 24 uniformly-distributed directions and updated at 40 Hz.

We used a spike-triggered “motion reverse correlation” analysis to compute the probability of each motion direction appearing in the 250 ms period preceding a spike. We show that direction tuning curves can be extracted by comparing the relative probability of each direction in the time window 50-100 ms preceding each spike. Tuning curves determined with this “reverse correlation” method are similar to those obtained using traditional “forward-correlation” approaches. Critically, across neurons, temporal integration windows vary in duration from 50-120 ms, with spiking probabilities affected up to 170 ms after stimulus presentation in some neurons.

We argue that the range of temporal integration windows observed neurons may provide a simple neuronal mechanism for balancing the demands of rapid versus reliable processing. Speed and accuracy can be traded-off by reading out the activity of different populations of neurons, without requiring individual neurons to adjust their synaptic integration properties.

(247 words)

POS-THU-184

CONSTITUTIVE EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) FAMILY LIGAND (GFL) RECEPTORS IN BONE AFFERENT NEURONS

Ringuet MT, Ivanusic JJ
Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Australia

Recent studies have suggested that Glial cell line-Derived Neurotrophic Factor (GDNF) Family Ligands (GFLs) are involved in the pathogenesis of pain. We are currently investigating the role of GFL signalling in bone pain. GDNF, Neurturin and Artemin bind to receptors GFRα1, 2 and 3, respectively. The aim of this study was to determine constitutive expression of GFL receptors (GFRα1, 2 and 3) in bone afferent neurons. Retrograde tracing was used to identify bone afferent neurons in male, Sprague Dawely rats. Double label immunohistochemistry was used to identify GFL receptors in retrograde labelled bone afferent neurons, and to define subpopulations that may be nociceptive. Antibodies were directed against GFRα1, 2 and 3 receptors, TRPV1 (to identify polymodal nociceptors), TrkA (to identify NGF-sensitive neurons) and CGRP (to identify peptidergic nociceptors). Almost all bone afferents were classified as small or medium sized (<1800μm^2). A substantial proportion of bone afferent neurons were GFRα3-immunoreactive (-IR) (36±11%; n=8). Most GFRα3-IR bone afferent neurons were TRPV1-IR (62±29%; n=3), TrkA-IR (75±10%; n=3) or CGRP-IR (62±18%; n=3), suggesting they are predominantly NGF-sensitive, peptidergic polymodal nociceptors. Many bone afferent neurons were GFRα2-IR (30±11%; n=8). Few of these were TRPV1-IR (15±10%; n=3), TrkA-IR (28±5%; n=3) or CGRP-IR (39±22%; n=3). Relatively few bone afferent neurons were GFRα1-IR (16±9%; n=8), and few of these were TRPV1-IR (12±11%; n=3), TrkA-IR (27±24%; n=3) or CGRP-IR (45±24%; n=3). Our findings suggest that bone afferent neurons express receptors required for GFL signalling in bone pain. (237 words).

POS-THU-185

PLASMA EXTRAVASATION STUDIES TO DETERMINE THE EXTENT OF LATERAL SPROUTING AFTER MEDIAN NERVE INJURIES

Shaikh S[1], Shortland P[2], Mahns DA[1]

1. School of Medicine, University of Western Sydney, Sydney, Australia. 2. School of Science and Health, University of Western Sydney, Sydney, Australia.

Previously, we demonstrated that following complete median nerve section, mechanical allodynia and thermal hyperalgesia developed bilaterally in distal paws within weeks of transection, i.e. well before the median nerve could have re-innervated the distal paw. In order to test whether the hyperalgesia resulted from (a) pre-existing ulnar nerve terminals in the median nerve area or (b) axonal sprouting from the ulnar branches into the median area (induced by nerve injury), we used anti-dromically evoked extravasation of Evans Blue to map the area of innervation by the median and ulnar nerves, and changes therein, before and after nerve injury. In female Long Evan rats, surgery was conducted under anaesthesia. The left median nerve was isolated, and cut, at the mid-axillar level. Ten minutes after nerve transection, a bolus dose of Evans Blue dye (50 mg/kg i.v.) was administered through jugular vein. The sectioned nerve was electrically stimulated at C-fibre strength for 5min (15V, 0.26Hz, 0.5ms). The regional distribution of Evan Blue extravasation in hairy and glabrous regions of forepaws was mapped and quantified by dye extraction (4% formaldehyde for 24h at 60°C) and spectro-photometric (620nm) measurement. Before nerve stimulation, no extravasation of dye was seen in the paws. In control experiments, nerve stimulation increased the dye concentration in the paws within the innervation territory of stimulated nerve. In adjacent regions innervated by unstimulated nerves in the forepaws, little dye extravasation was observed in comparison with stimulated nerve area. In future, the above experiment will be repeated in animals following nerve injuries. (250 words)

POS-THU-186

CORRELATED SPIKING ACTIVITY MIRRORS COHERENCE OF LOCAL FIELD POTENTIALS IN THE MIDDLE TEMPORAL (MT) AREA OF MARMOSET.

Solomon SS[1,2], Chen SC[1,2], Morley JW[3], Solomon SG[2,4].

1. School of Medical Science, The University of Sydney, NSW, Australia.
2. ARC Centre of Excellence in Vision Science, Australia.
3. School of Medicine, The University of Western Sydney, NSW, Australia.
4. Institute of Behavioural Neuroscience, University College London, United Kingdom.

Objective: Spiking activity of neurons in area MT is correlated, but the temporal structure of these correlations depends on the stimulus. Correlations are strong and fast for responses to moving gratings, but weaker for responses to moving dot fields. Here we ask whether neural rhythms in area MT also depend on the visual stimulus. Method: Local field potentials (LFPs) were measured from area MT in Sufentanil-anaesthetised marmosets (*Callithrix jacchus*) (n=5), using 96-channel electrode arrays. The power in the induced LFPs at single electrodes (n = 547), and the coherence in the LFPs between pairs of electrodes (n = 21,812), was calculated for frequencies between 0.3 – 250 Hz. Results: LFP response to dot fields was a sustained increase in power over a broad range of frequencies (~20 – 250 Hz). In contrast, coherence decreased in all frequency bands. LFP response to drifting gratings was also an increase in power, but over a narrow band of frequencies (30 – 100 Hz) and for a shorter period. Coherence also increased in a narrow band of frequencies ranging between 15 – 80 Hz. Conclusion: Dot fields and gratings produce different patterns of LFP response that correspond to the differences in correlated spiking activity. Reduced coherence for dot fields mirror the low spike correlations, while the increased coherence at high frequencies mirror the fast spike correlations for gratings. (220 words).

POS-THU-187

HOW MUCH DO WE SEE AND REMEMBER OUTSIDE OF FIXATIONS IN NATURAL VISION?

Kaunitz LN[1], Rowe EG[1], Tsuchiya N[1,2].

1- School of Psychology and Psychiatry; Faculty of Medicine, Nursing and Health Sciences; Monash University, 2- Decoding and Controlling Brain Information, Japan Science and Technology Agency, Chiyoda-ku, Tokyo, Japan
Naotsugu.Tsuchiya@monash.edu

When viewing a scene, we form an impression of the objects in the scene. Despite its vividness, it remains unclear how much we consciously perceive and remember about the individual objects. A dominant view in psychology is that this impression of a scene constitutes an illusion – we become conscious and remember only those objects that we attend to. An alternate explanation suggests that we perceive and remember many aspects of the scene, including unattended objects. We investigated this controversy in a free-viewing search task, employing a dual-task design. As a dummy task, subjects were instructed to search for a "target" face in a crowded natural scene. Upon completion of the search, subjects were presented with two faces. One of the faces served as the "probe" while the other as a distractor. Using a gaze-contingent method, we selected the "probe" from those faces that were fixated during the search while the distractor from those that were not fixated. We measured perception and memory of the probe face in a 2AFC+rating task, assessing objective performance (i.e., type-1 measure in the signal detection theory) and accuracy of metacognition (i.e., type-2 measure: correlation between subjective confidence and performance). We found that the longer subjects fixated on the probe, the greater their objective performance and the higher their confidence. However, fixation duration was independent from accuracy of metacognition. Our modeling analysis suggests that these results are generated based on common signals mixed with independent noises, implying that response selection and confidence are processed independently. (249 words)

POS-THU-188

EFFICACY OF SYNCHRONISED NEURONAL OSCILLATIONS IN DORSAL STREAM FEEDBACK WITHIN THE VISUAL PATHWAY OF NON-HUMAN PRIMATES

Vidyasagar TR[1], Mohan Y[1], Jayakumar J[1], Levichkina E[1]

[1]Department of Optometry and Vision Sciences, The University of Melbourne, Parkville, Australia.

Introduction: Synchronised neuronal oscillations have been suggested as the way one cortical area influences neuronal activity in another cortical region, e.g., with regard to top-down processing in the dorsal visual stream (Visual area V1-area MT-posterior parietal cortex) to mediate a spotlight of attention. We tested the hypothesis that synchronised oscillations in the low gamma range (20-40 Hz) are more effective for orthodromic activation of V1 neurones and additionally that such activation would be centred around a location on the orientation map and would spread across a number of orientation domains without a preponderance for any one orientation.

Methods: We imaged the neuronal response of the anaesthetised macaque V1 using optical imaging of intrinsic signals. We then homed in on the MT site corresponding to the visual field represented by the imaged V1 cortex and applied microstimulation at this site (20-500 microamp, 200 microsecond long biphasic pulses at 20, 30 or 100 Hz) while performing optical imaging.

Results: Low gamma frequency stimulation produced a region of activation (clipped to show only the 5% or 31% most active pixels) in V1 whose extent, superimposed on the map of orientation domain, showed activation across a number of orientation domains. There was no significant region of activation at 100 Hz over that same area of the V1 cortex.

Conclusions: Top-down activation from MT on area V1 occurs preferentially at low gamma frequencies and acts to ‘spotlight’ a location and has little feature selectivity. (238 words).

POS-THU-190

CHARACTERIZATION OF CALRETININ IMMUNOREACTIVE CELLS IN THE HUMAN RETINA.

Weltzien F[1], Lee SCS[1], Denkhaus C[1], Martin PR[1,2], Grünert U[1]

[1]Save Sight Institute and [2]School of Medical Sciences, University of Sydney, Sydney, Australia

In some mammalian retinas, the calcium binding protein calretinin is expressed by a variety of neurones including the AII amacrine cell, a crucial neuron in the rod pathway. Here we characterize the calretinin expressing neurones in human retina. Post mortem donor eyes were obtained with consent from the Lions' NSW Eye Bank. Vertical Vibratome sections were cut along the horizontal meridian and processed for calretinin immunofluorescence. Other pieces of retina were pre-labelled with antibodies specific for calretinin and subsequently immunopositive cells were injected with the lipophilic dye DiI. Calretinin immunoreactive cells were located in the inner nuclear layer (INL) or in the ganglion cell layer (GCL). A total of 67 DiI labeled cells (27 in INL, 40 in GCL) was analysed. Most of the cells in the INL (23/27, 85%) had AII type morphology. In addition, we identified a wide-field amacrine cell type (n = 9) with semilunar type morphology as described (Kolb et al., 1992). These cells were located either in the INL (n = 4) or in the GCL (n = 5). Three of the DiI injected cells in the GCL resembled the G17 ganglion cell (Kolb et al., 1992). Most of the other injected cells in the GCL, had large dendritic fields and lacked axons suggesting that they are were wide-field amacrine cells (stellate-varicose type, n=23). Antibodies to calretinin can be used to identify AII and other amacrine cells in the human retina, enabling quantitative analysis of these cell types. (243 words)

POS-THU-191

CONTRIBUTION OF TRPC3 CHANNELS TO BDNF-MEDIATED NEURITOGENESIS IN POSTNATAL MOUSE SPIRAL GANGLION NEURON EXPLANTS

Wong ACY[1], Ng A[1], Birnbaumer L[2], Housley GD[1]

[1]Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, the University of New South Wales, Sydney, Australia [2] Laboratory of Neurobiology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC27709, USA

Canonical transient receptor potential (TRPC) non-selective cation channels have been implicated in CNS neurite outgrowth in response to brain-derived neurotrophic factor (BDNF).

BDNF binding to TrkB receptors drives the phospholipase C_{γ} -PIP_2- diacylglycerol (DAG) second messenger pathway, activating TRPC3 channels. We examined the contribution of TRPC3 channels in BDNF mediated neuritogenesis in mouse spiral ganglion neurons (SGN) using a cultured SGN explant model. Postnatal day 5 SGN explants from the base, mid-region and apex of wildtype mouse cochleae (C129/SvEv background) and TRPC3 null mice (TRPC3KO) were cultured for 48 hours with BDNF (10 – 100 ng/ml) and the number of neurites emanating from the explants, the length of neurite extension and the number of secondary branches were assessed. Neurite outgrowth was identified by immunolabelling the explants with a rabbit polyclonal 200 kDa anti-neurofilament primary antibody and Alexa-594 conjugated secondary antibody, visualized by epifluorescence microscopy and analysed using Neuron J/Image J (NIH). With C129/SvEv SGN explants, all neuritogenesis parameters were enhanced by BDNF treatment, the strongest outgrowth was observed in the basal turn explants, for example, the cumulative neurite lengths in 100 ng/ml BDNF treated explants were 2.2x, 3.2x and 3.1x longer compared to the average of non-treated controls extracted from the base, mid, and apical cochlear turns ($p<0.001$, two-way ANOVA). BDNF-treated TRPC3KO SGN basal turn explants displayed significantly less neuritogenesis, with cumulative length 4.5x shorter compared to BDNF-treated C129/SvEv explants ($p<0.001$, Holm-Sidak comparisons, two-way-ANOVA). Our results support TRPC3 channels as an effector of BDNF-mediated neuritogenesis in mouse primary afferent auditory neurons. (250 words)

POS-THU-192

RESPONSE PROPERTIES OF V1 NEURONES IN THE FAR PERIPHERAL REPRESENTATION OF THE VISUAL FIELD

Yu HH[1], Rosa MGP[1,2]

1. Department of Physiology, Monash University, Clayton, VIC, Australia
2. Monash Vision Group, Monash University, Clayton, VIC, Australia

Although the primary visual cortex (V1) is one of the most extensively studied areas of the primate brain, very little is known about how the far peripheral visual space is represented in this area. We characterized the response properties of V1 neurones in anaesthetised marmoset monkeys, using high-contrast drifting gratings. We found that orientation selectivity of individual cells was similar from the centre (3-5° of eccentricity) to the far periphery (>50° eccentricity). Nonetheless, the proportion of orientation-selective neurones was higher in central visual field representation than in the peripheral representations (permutation test: $P<0.001$). In addition, there were similar proportions of cells representing all orientations, with the exception of the representation of the far periphery, where we detected a bias favouring near-horizontal orientations (Rayleigh test $R=0.2191$, $P=0.0002$). The proportions of direction-selective cells were similar throughout V1 (permutation test, $P=0.3441$). When the centre/surround organization of the receptive fields was tested with gratings with varying diameters, we found that the population of
neurones that was suppressed by large gratings was smaller in the far periphery (�2=16.5205, $P=0.0002$), although the strength of suppression in these cells tended to be stronger (Mann-Whitney $U=1313$, $P<0.0001$). In addition, the ratio between the diameters of the excitatory centres and suppressive surrounds was similar across the entire visual field (Mann-Whitney $U=3112$, $P=0.612011$). These results suggest that, superimposed on the broad uniformity of V1, there are subtle physiological differences, which indicate that spatial information is processed differently in the central versus far peripheral visual fields. (245 words)

POS-THU-193

STIMULUS ESTIMATES FROM NEURONAL DECODING EXCEED PERCEPTUAL PERFORMANCE

Zavitz E, Haghgooie S, Yu HH, Davies AJ, Rosa MGP, Price NSC
Department of Physiology, Monash University, Clayton, Victoria, Australia

A fundamental role of sensory neurons is to facilitate accurate and rapid perception of the properties of a continually changing environment. It remains unclear how populations of neurons with diverse tuning properties encode motion direction, and the timescales over which accurate estimates of stimulus properties can be decoded from neural activity.

We recorded single- and multi-unit neuronal activity from 96 electrode “Utah” arrays implanted in the middle temporal area (MT) of the anaesthetised (sufentanil, 8µ/kg/h, and N2O, 70% in oxygen) marmoset (*Callithrix jacchus)*. Neural responses were recorded to a dot stimulus that moved coherently in a single direction. Stimulus directions were chosen randomly from 24 uniformly-distributed directions and updated at 40 Hz.

To examine the encoding of rapidly changing direction information across the population of MT neurons, we used linear discriminant analysis to classify stimulus direction from sliding windows of 35 ms of neural activity. Following training, the classifier was able to decode stimulus direction with an accuracy of 15-20% (chance performance is 4.2%) across three different speeds. Overall, 57-67% of trials were accurate within 30˚ (2 possible direction bins) of the presented stimulus.

Our results demonstrate that information about rapidly changing (40 Hz) stimuli can be resolved from neuronal activity, even though our psychophysical studies suggest that conscious perception of motion direction does not occur following such brief exposures. (220 words).

POS-THU-194

BINOCULAR RECEPTIVE FIELDS IN THE MARMOSET LATERAL GENICULATE NUCLEUS

Zeater N[1,2], Cheong SK[1,2], Solomon SG[1,3], Dreher B[3], Morley JW[4,5], Focke CMB[6], and Martin PR[1,2,3]

[1]ARC Centre of Excellence in Vision Science, The University of Sydney. [2]Save Sight Institute, The University of Sydney. [3]School of Medical Sciences, The University of Sydney. [4]School of Medicine, The University of Western Sydney. [5]School of Medical Sciences, The University of New South Wales. [6] Faculty of Biology and Psychology, University of Göttingen.

Purpose: Each layer of the lateral geniculate nucleus of the dorsal thalamus (LGN) receives dominant excitatory input from one eye. Previous studies have shown that stimuli presented through the non-dominant eye can modify (usually, suppressively) background activity and responses to dominant eye stimulation. Here we describe a population of receptive fields in LGN of New-World, marmoset monkeys (*Callithrix jacchus*) that receive binocular excitatory inputs.

Methods: Extracellular recordings of neuronal activity were made in the LGN of 3 Sufentanil-anaesthetised adult marmosets. Recordings were made with a NeuroNexus 32-channel probe comprising two shanks horizontally separated by 0.5 mm; each shank has 16 recording points vertically separated by 0.05 mm. Single-cell activity in response to pulsed achromatic and cone-isolating visual stimuli was recorded. The visual stimuli were presented to each eye sequentially, or binocularly. In 2 animals, recording sites in the LGN were verified anatomically.

Results: We characterised responses of 110 cells. Of these cells 54 (49%) were unaffected by non-dominant eye stimulation and 21 (20%) were weakly suppressed or excited. About a quarter (29 cells; 26%) showed almost identical responses to stimulation of either eye. These ‘binocular’ responses were most common in ON-OFF (12/14) and suppressed-by-contrast (5/5) receptive field classes.

Conclusions: Some (mostly non-standard) receptive fields in marmoset LGN receive binocular inputs. Their response properties and recording location suggest association with the koniocellular subdivision of the retino-geniculate visual pathway. Whether the site of binocular convergence is in the LGN or elsewhere in the visual system was not established by our experiments.

(Word count: 249)

POS-THU-195

ALTERED GABAA RECEPTOR α1 SUBUNIT PROTEIN EXPRESSION FOLLOWING LOW DOSE PRENATAL ETHANOL EXPOSURE IN THE NEONATAL AND ADULT RAT CEREBELLUM

Cullen CL[1], DeGasperi S[1], Miller SM[2], Moritz KM[1], Bjorkman ST[2].

[1]School of Biomedical Science, The University of Queensland, St. Lucia, Brisbane, Queensland 4072

[2]The University of Queensland Centre for Clinical Research, Herston, Brisbane, Queensland 4006

Excessive alcohol consumption during pregnancy can lead to a wide range of long term structural and functional deficits in the developing brain, including alterations to GABA signalling and the $GABA_A$ receptor. However, little is known about the long term consequences on the GABAergic system following low level alcohol consumption during pregnancy. The aim of this study was to investigate the expression of the $GABA_A$ receptor subunits a1 and a3 which dominant during adulthood and development respectively, following low dose prenatal alcohol exposure. Pregnant Sprague-Dawley rat dams were fed an ad libitum diet containing a low dose of ethanol (6% v/v, EtOH) or an isocaloric control diet (Control) for the duration of pregnancy. Cerebellar tissue was collected from male and female offspring at PN1 and 8 months (n=8-10 per group). Protein expression of the $GABA_A$ receptor α_1 and α_3 subunits was determined using SDS-PAGE western blotting. Male EtOH offspring expressed significantly lower levels of the α_1 subunit protein *cf.* control offspring at PN1 and 8 months of age. The α_3 subunit was not expressed in 8 month cerebellar tissue of control or EtOH offspring and there was no significant effect of prenatal treatment on protein expression of this subunit at PN1. These findings demonstrate that consumption of even relatively small amounts of alcohol can lead to long term alterations to the $GABA_A$ receptor which may lead to altered receptor pharmacology and function. (231 words)

POS-THU-196

PROTECTIVE POTENTIAL OF 17-BETA-ESTRADIOL ON MEMBRANE LINKED FUNCTIONS IN AGING FEMALE RATS: A BEHAVIORAL, BIOCHEMICAL AND ULTRASTRUCTURAL STUDY

Kumar P[1], Kale R[1] and Baquer N[1]

[1]School of Life Sciences, Jawaharlal Nehru University, New Delhi, 110067, India

Objective: The objective of this study was to investigate neuroprotective potential of 17β estradiol (E2) treatment on the activities of acetylcholinesterase and monoamine oxidase, membrane fluidity , neurolipofuscin, genomic DNA degradation, protein oxidation levels and testing learning memory, occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol.

Background: Alzheimer's disease (AD) is the most common form of dementia in the elderly. AD is characterized by the presence of amyloid plaques which are formed from deposits of b- amyloid protein (Ab). These changes increase during menopausal condition in females when the level of estradiol is decreased.

Methods: The aged rats (12 and 24 months old) (n= 8 for each group) were given subcutaneous injection of 17β -estradiol (0.1 µg/g body weight) daily for one month. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and brains were isolated for further study. Learning was tested in a Morris water maze and ultrastructural studies of brain region by MRI.

Results: The results obtained in the present work revealed that normal aging was associated with significant increases in the activity of monoamine oxidase, genomic DNA degradation and protein oxidation levels in the brains of aging female rats, and a decrease in acetylcholinesterase activity and membrane polarization. Ultrastructural studies of the frontal cortex of exposed rats revealed that the changes were more pronounced in the aged treated rats in terms of presence of lipofuscin, vacuolization and lysosomal degradation. Our data showed that exogenous administration of E2 brought these changes to near normalcy in aging female rats.

Conclusions: It can therefore be concluded that E2's beneficial effects seemed to arise from its, antioxidant and antilipidperoxidative effects, implying a therapeutic potential drug for age related changes. Based on our studies and others, we conclude that E2 have therapeutic potential for adjunctive therapy for the AD.

POS-THU-197

AXONAL FUSION IN REGENERATING AXONS SHARES MOLECULAR COMPONENTS WITH THE APOPTOTIC CELL RECOGNITION PATHWAY.

Neumann B[1], Coakley S[1], Giordano-Santini R[1], Linton C[1], Zhang Y[2], Yang H[3], Xue D[2,3], Hilliard MA[1].

1. Queensland Brain Institute, The University of Queensland, Brisbane QLD, Australia. 2. School of Life Sciences, Tsinghua University, Beijing 100084, China. 3. Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder CO, USA.

Understanding the molecular mechanisms regulating axonal regeneration is essential for the development of effective therapies for nerve injuries. However, we have a very poor understanding of how target reconnection occurs. Previously, we and others have demonstrated that target reconnection in *C. elegans* severed mechanosensory neurons can occur through a process of axonal fusion, with the proximal regrowing fragment recognizing and re-establishing membrane and cytoplasmic continuity with its own separated distal fragment. We have now characterised the process of axonal fusion at the molecular level, uncovering a critical role for molecules previously shown to mediate the recognition of apoptotic cells by neighbouring phagocytes. We have discovered that in animals carrying mutations in the conserved apoptotic phosphatidylserine receptor gene, *psr-1*, the proximal axon regenerates and contacts the distal fragment, but is unable to fuse, as a result of which the distal fragment degenerates. PSR-1 has previously been shown to bind exposed phosphatidylserine (PS) on the surface of apoptotic cells and we find that PS is also exposed on the severed axon where it functions as a “save-me” signal for recognition by the regrowing axon. Furthermore, we demonstrate that PSR-1 functions in the same pathway as the secreted transthyretin-like TTR-52, which binds to exposed PS after transection, the phagocyte receptor CED-1, and the intracellular adaptor phosphotyrosine-binding protein CED-6. We propose that PSR-1, CED-1, and CED-6 function cell-autonomously, while TTR-52 is expressed from the intestines, from where it binds and mediates the recognition between the regrowing axon and its distal fragment. (247 words).

POS-THU-198

LOSS OF MEC-17 LEADS TO MICROTUBULE INSTABILITY AND AXONAL DEGENERATION

Neumann B, and Hilliard MA
Queensland Brain Institute, The University of Queensland, Brisbane QLD, Australia.

Maintenance of axonal structure is critical for neuronal function, and axonal degeneration is a hallmark for a number of neurodegenerative diseases. Despite the depth of knowledge that has emerged about how axons are maintained and how they degenerate, only a handful of genes that trigger axonal degeneration have been identified. From forward genetic screening in *C. elegans*, we have identified the α-tubulin acetyltransferase gene *mec-17* as causing spontaneous, adult-onset, and progressive axonal degeneration. MEC-17/αTAT1 was recently identified as an α-tubulin acetyltransferase enzyme conserved across all ciliated organisms, and found to be critical for microtubule number and organization. We demonstrate that MEC-17 is essential for maintenance of axonal structure in the mechanosensory neurons of *C. elegans*, with mutants displaying degeneration of the axon in up to 45% of adult animals. Loss of MEC-17 led to microtubule instability, a reduction in mitochondrial number, and disrupted axonal transport, with altered distribution of both mitochondria and synaptic components. Furthermore, our results demonstrate that *mec-17*-mediated axonal degeneration occurs independently from its acetyltransferase domain, is enhanced by mutation of the tubulin-associated molecule COEL-1, and is strongly influenced by the animal's body length. This study therefore identifies a critical role for the conserved microtubule-associated protein MEC-17 in preserving axon integrity and preventing axonal degeneration. (207 words).

POS-THU-199

PREDICTING STOCHASTIC PERCEPTUAL FLUCTUATION IN CONTINUOUS FLASH SUPPRESSION IN EEG

Paton, B[1, 2, 3], Hohwy, J[2] and Tsuchiya N[1,4]

1. School of Psychology and Psychiatry, Monash University
2. Philosophy & Cognition Lab, SOPHIS, Monash University
3. Monash Biomedical Imaging, Monash University
4. Decoding and Controlling Brain Information, Japan Science and Technology Agency, Chiyoda-ku, Tokyo, Japan

Prediction of future choices and perceptual decisions is possible based on biological signals such as EEG, fMRI and pupil dilation. Taking advantage of multivariate decoding techniques, recent studies have reported reliable prediction of perceptual decisions several seconds before a response. Some of these predictions are based on the intrinsic neuronal fluctuations before presentation of the stimulus. It is unclear exactly how these pre-stimulus neuronal fluctuations are linked to stochastic perceptual fluctuation during perception of ambiguous stimuli. To investigate the putative neural-perceptual link, we measured EEG (64 channels, n = 24) in subjects who observed a dynamic Continuous Flash Suppression (CFS) experiment. Using CFS, we suppressed a face from conscious awareness and tried to identify any EEG response that predicted the timing of the break from suppression of the face.

Using a linear kernel Support Vector Machine (SVM) multivariate decoder we were able to distinguish reliably trials where suppression broke quickly from those where it lasted for a longer duration. This was based on the EEG response up to 1.5 seconds before the onset of the to-be-suppressed face. To distinguish the timing of perceptual switches on a trial-by-trial basis, we found that the most informative EEG response features were changes in the phase of the low frequency bands (5-10Hz) of the EEG signals. These signals originate from bilateral occipital and early temporal electrodes. Our results suggest that timing of low-frequency activity in visual areas even before stimulus onset contains a great deal of information relevant to much later perceptual switches. (249 words)

POS-THU-200

PREDICTING STIMULUS VISIBILITY BASED ON PRE-STIMULUS EEG RESPONSES

Mana Fujiwara(1,3), Riccarda Peters(1,3), Roger Koening(1) and Naotsugu Tsuchiya (1,2)

1. School of Psychology and Psychiatry, Monash University
2. Decoding and Controlling Brain Information, Japan Science and Technology Agency, Chiyoda- ku, Tokyo, Japan
3. Equal contribution

CFS (Continuous Flash Suppression) is a powerful visual illusion, which enables us to dissociate subjective awareness from physical visual input. In CFS a stationary stimulus is presented into one eye while colourful random Mondrian patterns are flashed into the other eye at a rate of 10Hz. In most trials, Mondrians dominate percept, but, in some trials, the suppressed stimulus can break through Mondrians. The exact reasons why the effectiveness of CFS varies across trials remain unclear. Here, we hypothesized that pre-stimulus oscillations, specifically in the alpha range (~10 Hz), may determine the effectiveness of the strength of 10Hz CFS. To test this hypothesis, we recorded EEG from human subjects (n = 8) while they tried to detect a Gabor patch that was located in one of the four quadrants (i.e., a 4AFC localization task). The Gabor was weakly suppressed by low-contrast CFS, resulting in ~50% correct detection rates. In each trial, subjects rated visibility of the Gabor from 1 to 4. As we hypothesised, we found that inter-trial coherence of phase (e.g. argument of Fourier Transform) of low frequency EEG components distinguished perceptual outcomes (i.e., correct vs incorrect, different visibility ratings) of the upcoming Gabor. We localized the difference to originate from the occipital lobe and -750 to 250 ms from the CFS onset. Our findings suggest that trial-by-trial fluctuation of percepts in CFS is predictable from the relationship between the timing of the stimulus onset and the ongoing low-frequency EEG. (Need to be less than 250 words)

POS-THU-201

THE NEURONAL MECHANISMS OF STEADY STATE VISUALLY EVOKED POTENTIAL (SSVEP) STUDIED IN THE FLY BRAINS WITH MULTI-CONTACT ELECTRODES

Dror Cohen[1#], Angelique Christine Paulk[2], Bruno Van Swinderen[2] and Naotsugu Tsuchiya[1]
[1] School of Psychology and Psychiatry, Monash University, Australia[2]
[2] Queensland Brain Institute, The University of Queensland, Australia

Introduction

Flickering stimuli that induce Steady State Visually Evoked Potentials (SSVEP) have been successfully used to study cognitive processes in humans and animals. In particular, covert shifts of attention have been shown to enhance the SSVEP responses for the attended flickers and to reduce those for the ignored flickers. Surprisingly, homologous attentional modulation has been reported in fruit flies using SSVEP. The SSVEP, combined with the genetic manipulations available for flies, opens up possible dissection of high-level cognitive processes such as attention at the neuro-circuit level. Here, we investigated the neuronal mechanisms of SSVEP responses in flies using electrophysiological recording techniques and machine learning.

Methods

We recorded local neural activity (Local Field Potentials, LFP) in the brains of fruit flies (Drosophila Melanogaster), using a 16-channel micro linear array inserted laterally across the fly brain. During recording, a flicker stimulus was presented either at the left, right or the both sides of the visual field. The flicker frequency was either 13Hz or 16Hz. We characterised the physiological response properties of SSVEP by analysing the LFPs in time and frequency domains. To quantify the information about the stimulus represented in the fly brain, we used a multi-variate decoding technique; specifically, we used a linear classifier (Support Vector Machine) to quantify when, where, and at which frequencies, the information about the flickers was encoded in the LFPs.

Results

The analysis in time and frequency revealed precise and consistent responses to the flickers in the peripheral visual systems. However, significant responses were also observed in central brain areas. Most notably, in addition to the expected SSVEP response at the flicker frequencies we observed strong responses at the harmonics (up to 7th harmonic) and inter-modulatory frequencies (e.g., 29Hz = 13Hz+16Hz). Decoding analyses confirmed highly localized information both in space and frequency and nearly perfect accuracy in classifying the stimuli.

Discussion

Our initial step towards understanding SSVEP in files is highly promising. Ongoing experiments will test the neuronal basis of attentional modulation using various paradigms. For example, we will make one flickering stimuli more salient by pairing it with reward/punishment. By combining with genetic manipulations of local neural circuits, we will extend our analysis to quantify how information about the attended and ignored flickers is represented in the brains.

POS-THU-202

ELECTRICAL POPULATION BEHAVIOUR OF LAYER 2/3 PYRAMIDAL NEURONS REVEALED BY A GENETICALLY ENCODED VOLTAGE INDICATOR (VSFP-Butterfly) IN MOUSE MOTOR CORTEX

Empson RM[1], Goulton, C[1], Sears, K[1], Zeng, H[2], Knöpfel, T[3]

Department of Physiology, Brain Health Research Centre, University of Otago, Dunedin, New Zealand[1]; Allen Institute for Brain Science[2], Optogenetics and Circuit Neurosciences, Division of Brain Sciences, Imperial College London, London W12 0NN, UK[3]

The ability to monitor the electrical behaviour of groups of uniquely identified, and connected, neurons is a major goal if we are to understand how the brain works. Towards this goal we have developed molecular technology that harnesses light to measure voltage[1] and coupled this with an advanced genetic targeting strategy. The targeting strategy aimed to express the FRET-based voltage indicator, VSFP Butterfly, specifically in layer 2/3 (L2/3) of cortical pyramidal neurons using ras-grf2[2] and CaMkinaseII regulatory sequences. Confocal microscopy confirmed very high levels of VSFP Butterfly expression in the plasma membrane of layer 2/3 neurons across the cortex in these mice. High speed (100Hz) live fluorescence imaging with simultaneous classical stimulation and electrical recording revealed fast, robust changes in fluorescence from L2/3 neurons in motor cortex. In response to a single L5 stimulation that evoked a small, synaptic local field potential, the fluorescence transients in L2/3 peaked within 10 ms with a duration of 300-400 ms (n = 5 mice). We routinely observed a decrease in the FRET donor (citrine) signal concomitant with an increase in the FRET acceptor (mKate) signal. These signals provide a real-time, ratiometric voltage signal from all neurons within the field of view to generate a content-rich activity map. This exciting new transgenic mouse tool brings a modern and specific approach to voltage imaging in the brain. It also provides a powerful optogenetic reporter approach that will help untangle the complexities of cortical microcircuit dynamics.

1. Akemann *et al* Nat Methods. 2010 Aug;7(8):643-9.
2. http://www.brain-map.org/

POS-THU-203

EFFECT OF PROBDNF ON ENDOTHELIUM AND ANGIOGENESIS

Li H[1,2], Liu J[1], Lim Y[1], Saadipour K[1], Zhong JH[1], Yang M[1], Zhou XF[1]

1. School of Pharmacy and Medical Sciences, Division of Health Sciences, University of South Australia, Adelaide, SA. 2. Department of Histology and Embryology, Hunan University of Traditional Chinese Medicine, Changsha, Hunan Province, China, 410208.

Brain derived neurotrophic factor (BDNF) is a member of neurotrophin family which is best characterized for its survival and differentiative effects on neurons. Recent studies indicate that, in addition to its neuropoietic actions, BDNF shows functions on promoting endothelial cell survival and inducing angiogenesis in physiology and ischemic tissues through trkB receptor [1]. ProBDNF is the precursor of BDNF which has different functions from mature BDNF. In this study, we investigated the effects of ProBDNF on the viability and angiogenesis using human umbilical vein endothelial cells (HUVEC) as a model. ProBDNF and its receptors can be detected in HUVEC. MTT results show proBDNF can decrease HUVEC viability from 3ng/ml and this effect can be blocked by anti-proBDNF. HUVEC tube forming function (angiogenesis) was significantly inhibited by ProBDNF (30ng/ml). The tube area in proBDNF group is obviously less than control group. This study suggests proBDNF plays an inhibitor role in endothelium and angiogenesis. Further study on proBDNF in angiogenesis may lead to a new insight into the treatment of ischemic injury or cancer.

POS-THU-204

ONGOING BRAIN ACTIVITY IN SUBJECTS WITH CHRONIC OROFACIAL PAIN MEASURED BY QUANTITATIVE ARTERIAL SPIN LABELING

Youssef AM[1], Reeves JM[1], Nash PJ[1], Gustin SM[1], Petersen ET[2], Peck CC[3], Murray GM[3], Henderson LA[1]

1Department of Anatomy and Histology, University of Sydney, Sydney, NSW 2006, Australia. 2Departments of Radiology and Radiotherapy, University Medical Center Utrecht, The Netherlands. 3Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry, University of Sydney, Westmead Hospital, Westmead, NSW 2006, Australia.

Over the past decade, human brain imaging investigations have revealed that chronic pain conditions are associated with changes in higher brain structures. Chronic neuropathic pain has been shown to be almost exclusively associated with decreased activity in the thalamus, whereas non-neuropathic pain is typically associated with increased co-activation of numerous brain regions, including the thalamus, prefrontal cortex, insular and cingulate cortices. The appreciation of any differences is important since better treatment development will depend on understanding the underlying mechanisms of different forms of chronic pain. The aim of this investigation was to use an MRI technique, quantitative arterial spin labeling (qASL), to compare and contrast regional cerebral and brainstem blood flow in subjects with chronic neuropathic orofacial pain (painful trigeminal neuropathy; PTN) and in individuals with chronic non-neuropathic orofacial pain (painful temporomandibular disorders; TMD). Eighteen subjects with PTN, 15 subjects with TMD and 54 pain-free controls were recruited for the study. In PTN subject, qASL revealed significant CBF decreases in the thalamus, primary somatosensory cortex, cerebellum and in the region of the ascending trigeminothalamic tract. In contrast, TMD subjects were associated with blood flow increases in brain regions code the emotional and cognitive aspects of acute pain, such as the anterior cingulate, and dorsolateral prefrontal cortices. Furthermore, TMD was associated with increased CBF within motor-related brain regions, such as well as within the caudalis division of spinal trigeminal nucleus. Our data reveals that neuropathic and non-neuropathic orofacial pain are associated with different patterns of resting regional brain activity. (248 words).

POS-THU-205

FUNCTIONAL RECOVERY OF HIPPOCAMPAL SYNAPTIC TRANSMISSION FOLLOWING GLUTAMATE EXCITOTOXICITY.

Gorlamandala N, Craig AJ, Krishnan AV, Power J, Housley GD

Translational Neuroscience Facility, Department of Physiology, School of Medical Sciences, University of New South Wales, Australia.

We are investigating the functional recovery of synaptic transmission in an *in vitro* model of glutamate excitotoxicity. Elevated levels of extracellular glutamate and glutamate excitotoxicity have been implicated in the neuronal damage associated with ischemia .Glutamate excitotoxicity is typically assayed using either in vivo or in cell culture preparations; neither of which are well-suited to examine the loss of synaptic function associated with ischemia. While the brain slice preparation is well-suited to study synaptic function, the utility of the preparation as in an *in vitro* model of glutamate excitotoxicity is unclear. We examined synaptic transmission in the hippocampus of mice (C129 SvEv) brain slices (~5 week) as a functional indicator of neuronal vulnerability to excitotoxicity. Glutamate excitotoxicity was induced by addition of exogenous glutamate (1 mM, 50 min) to superfused slices. Schaffer collateral-CA1 neuron transmission recordings were carried out to assess the synaptic function, through excitatory postsynaptic field potential (fEPSP) recordings. Glutamate treatment produced a significant reduction in the slope of fEPSP (mean change = 47%; $P < 0.01$; paired t-test; n =8). Reperfusion of standard perfusate resulted in 80% recovery of synaptic potential, which was significantly different from the reduced fEPSP due to glutamate excitotoxic insult ($P = 0.02$; paired t-test; n = 8). This model has proven to be a robust indicator for functional recovery following an excitotoxic insult and will be used in further studies with knockout mice models to understand the role and contribution of specific ion channels in synaptic transmission under excitotoxic conditions. (248 words)

POS-THU-206

PEPTIDE DRUG SCAFFOLDS FOR TARGETING NEUROLOGICAL DISEASES

Wang CK[1], Craik DJ[1].

1. The Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

Cyclic peptide drugs are gaining increasing interest as a drug design framework owing to their favourable pharmacokinetic properties over small molecule compounds, such as increased specificity and lower toxicities. Here, we focus on naturally occurring disulfide-rich peptide scaffolds that have demonstrated remarkable stability against various enzymatic and chemical challenges. We propose that these frameworks are valuable drug design templates for neurological diseases. For example, our lab has synthesised a cyclic form of chlorotoxin, a peptide from Scorpion venom, which was conjugated to Cy5.5 and was used as an imaging agent to locate brain tumour cells and distinguish them from normal cells. We have chemically modified kalata B1, a cyclic peptide from a Coffee plant, to develop stable peptides for the treatment of multiple sclerosis, an inflammatory disease of the central nervous system. Peptide sequences from the $MOG_{35–55}$ epitope were grafted onto the peptide scaffold. One of the grafted peptides, MOG3, was able to substantially reduce both clinical and histological signs of experimental autoimmune encephalomyelitis in a mouse model. We are currently using these peptide drug frameworks to design novel drug leads for the treatment of Alzheimer's disease using a combined approach of combinatorial screening and molecular grafting. (198 words)

POS-THU-207

CHANGES IN $GABA_A$ RECEPTOR α_1 α_2 α_3 SUBUNIT EXPRESSION FOLLOWING NEONATAL HYPOXIA-ISCHAEMIA IN THE NEWBORN PIGLET.

Miller SM, Ireland Z, Sullivan SM, Colditz PB, Bjorkman ST.

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

Purpose: The principal function of the GABA system in mature brain is inhibition, however in the neonatal brain GABA provides much of the excitatory drive. Whilst anticonvulsants augment GABA's inhibitory actions in mature brain, administration of GABAergic drugs to neonates may exacerbate seizures and worsen brain injury. The current anticonvulsants are largely ineffective in neonates. Differences in $GABA_A$ receptor subunit composition will influence receptor pharmacology. We aimed to assess changes in expression of the $GABA_A$ receptor α-subunits in the neonatal HI piglet.

Methods: Piglets (n=34) anaesthetised, ventilated, catheterised and subjected to 30min HI, controls (n=13) underwent anaesthesia without HI. Animals were euthanased at 24 or 72h post-insult. HI animals were grouped based on presence/absence of seizure activity. Brain tissue from seven regions of interest was collected and $GABA_A$ receptor α_1 α_2 and α_3 protein expression was analysed by western blot and immunofluorescence.

Results: $GABA_A$ α_1 and α_3 protein expression was altered temporally and regionally following HI. Significant reductions in α_1 and α_3-subunit were observed in HI-seizure animals at 72h. Immunofluorescence revealed α_3 labelling restricted to the cytoplasm, with α_1 labelling localised to the cell membrane also. α_2-subunit expression was unaltered by neonatal HI.

Conclusions: $GABA_A$ receptor α_1 and α_3-subunit expression was decreased following neonatal HI; presence of seizures further reduced this expression. The localisation of α_1 to the cell membrane suggests this may be a membrane-bound functional subunit and a potential therapeutic target for novel anticonvulsant development. (237 words)

POS-THU-209

DRUGS HAVE LITTLE SWEET APPEAL: GREATER ATTRACTION TO AND PREFERENCE FOR SWEET VERSUS DRUG CUES.

Madsen HB[1,2,3], Ahmed SH[1,2]

1. Université de Bordeaux, Institut des Maladies Neurodégénératives, Bordeaux, France. 2. CNRS, Institut des Maladies Neurodégénératives, Bordeaux, France. 3. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australia

Despite the unique ability of addictive drugs to directly activate brain reward circuits, recent evidence suggests that drugs induce reinforcing and incentive effects that are comparable to, or even lower than some nondrug rewards. In particular, when rats have a choice between pressing a lever associated with intravenous cocaine or heroin delivery and a different lever associated with sweet water delivery, most respond on the latter. This outcome suggests that the sweet lever has acquired greater conditioned incentive value than the drug lever. However, this outcome may also be due to the differential ability of sweet versus drug levers to elicit Pavlovian feeding-like conditioned responses that cause the lever to deflect. To test this hypothesis, rats were first conditioned to associate one lever with sweet water delivery (0.2% saccharin) and a different lever with intravenous cocaine (0.25 mg) or heroin (0.01 mg) delivery. Choice between these two levers was then assessed under two discrete-trials choice procedures: one that permitted the expression of conditioned lever press responses at the moment of choice, and one that did not. As expected, during conditioning, rats approached and pressed the sweet lever considerably more than either drug lever and pressed the heroin lever slightly more than the cocaine lever. Importantly,though these differences in conditioned behavior predicted subsequent preference for sweet water during choice, they were not required for its expression. Overall this study shows that rats prefer the sweet lever during choice because it has acquired more conditioned incentive value than either drug lever. (250 words).

POS-THU-210

14-3-3ZETA MAINTAINS PHOSPHORYLATION OF NDEL1 TO CONTROL NEURAL MIGRATION

Eiman Saleh[1], H Ramshaw[1], P-S Cheah[1], X Xu[1], AF Lopez[1], & Q Schwarz[1]

[1]*Centre for Cancer Biology, SA Pathology, Adelaide, South Australia.*
[2] *Adelaide University of South Australia, Adelaide, South Australia*

Neuropsychiatric disorders such as schizophrenia are likely caused by a large number of genes with a small effect. Primarily they have complex traits believed to arise from multiple deficiencies within connected biological networks controlling neuronal migration, axonal pathfinding and synapse formation. Our lab has recently shown that 14-3-3zeta mouse mutants display deficits reminiscent to schizophrenic patients such as severe capacity to learn and remember, hyperactivity and disrupted sensorimotor gating. Developmental abnormalities of the hippocampus were also in the mutant mice.

My research is focused on determining whether 14-3-3zeta plays a role in the neural migration pathway causing aberrant neuronal migration observed previously in these mice. I have shown that phosphorylated Ndel1, a key molecule in neuronal migration, levels are reduced in the 14-3-3zeta mouse mutants and thus propose that 14-3-3zeta binds to the CDK5 phosphorylated Ndel1 to promote interaction with LIS1 and thereby promote neuronal migration. To test this model I have developed an in vitro migration assay with neural stem cells. Our in vitro model provides an ideal system to dissect the molecular mechanism in the neural migration pathway. Consistent with my finding that Ndel1 is aberrantly phosophorylated in 14-3-3zeta mutants, the in vitro migration assay revealed severe migration defects in the 14-3-3ζ deficient neurons compared to wild-type. These findings impersonate the LIS1 and NDEL1 deficient neurons which displayed severe and mild migration defects, respectively and thereby supports our proposed model. Furthermore, I have also found that the nuclear-centrosome coupling during migration is perturbed in vitro. These findings suggest that defects in coupling may contribute to migration defects in the 14-3-3zeta mutants.

POS-THU-211

MOUSE EFFERENT VESTIBULAR NEURONS SHOW HOMOGENOUS PASSIVE MEMBRANE AND DISCHARGE PROPERTIES IN VITRO

Mathews M, Wijesinghe R, Tung V, Zhang L, Camp A
School of Medical Science, Sydney Medical School and Bosch Institute, University of Sydney, Sydney, Australia

Impaired GABA-mediated neurotransmission has been implicated in many neurologic diseases, including epilepsy, intellectual disability and psychiatric disorders. We found that inhibitory neuron transplantation into the hippocampus of adult mice with confirmed epilepsy at the time of grafting markedly reduced the occurrence of electrographic seizures and restored behavioural deficits in spatial learning, hyperactivity and the aggressive response to handling. In the recipient brain, GABA progenitors migrated up to 1,500 μm from the injection site, expressed genes and proteins characteristic for interneurons, differentiated into functional inhibitory neurons and received excitatory synaptic input. In contrast with hippocampus, cell grafts into basolateral amygdala rescued the hyperactivity deficit, but did not alter seizure activity or other abnormal behaviours. Our results highlight a critical role for interneurons in epilepsy and suggest that interneuron cell transplantation is a powerful approach to halting seizures and rescuing accompanying deficits in severely epileptic mice. (144 words).

POS-THU-212

DEVELOPMENT AND CHARACTERIZATION OF TWO AUTOREGULATORY GENE EXPRESSION SYSTEMS FOR GENE THERAPY IN HUNTINGTON'S DISEASE MODEL

Wagner T[1, 3], Young D[1, 2, 3], Mouravlev A[2, 3].

1. Department of Molecular Medicine and Pathology. 2 Department of Pharmacology and Clinical Pharmacology.
3. Centre for Brain Research, University of Auckland, Auckland, New Zealand

The ability to regulate the expression of genes with therapeutic potential remains one of the key issues limiting clinical application of gene therapy. To address the unmet need for a regulatory gene expression system, we developed two novel autoregulatory gene expression cassettes. Within these cassettes, transgene expression is driven by either a transcription factor (ARF5), or a recombination enzyme (Cre) via their respective response elements in response to cell stress-induced activation of caspase-3, or calpain. The ARF5/Cre is fused with a dominant nuclear export signal (NES) via a linker containing the caspase-3/calpain cleavage site. Under basal conditions, the NES restricts ARF5/Cre to the cytosol, but when caspase-3/calpain is activated, NES is cleaved off from the ARF5/Cre, allowing it to translocate to the nucleus to drive expression of the transgene. Thus, this is a fully self-regulating system in which transgene expression is tightly coupled to cell stress. Using a GFP reporter gene, we analyzed functionality of the regulatory cassettes *in vitro* in response to neurotoxin-induced cell stress, or following co-expression with pathogenic proteins. We observed up to a 5-fold increase in GFP expression with the ARF5 cassette by Western blot analysis, but significantly lower basal GFP expression with the Cre cassette. The functionality of the Cre cassette was further examined *in vivo*, in a rat model of Huntington's disease. We showed that the regulatory cassettes are highly sensitive to activation of caspase-3/calpain in response to a variety of stress stimuli and may be a promising tool for application in gene therapy. (250 words)

POS-THU-213

DEVELOPING *IN VIVO* ASSAYS FOR INVESTIGATING P75^{NTR} AND NRH1 RANSMEMBRANE CLEAVAGE EVENTS USING ZEBRAFISH EMBRYOS

Jayne T[1], Lardelli M[1].

1. Zebrafish Genetics Laboratory, School of Molecular and Biomedical Science, The University of Adelaide, South Australia, Australia.

γ-secretase is an important protease complex as it is responsible for the cleavage of over 70 substrates within their transmembrane domains. γ-secretase plays a role in Alzheimer's disease through its processing of the AMYLOID PRECURSOR PROTEIN to produce the aggregation-prone Amyloidβ peptide. It is still not clear what determines γ-secretase cleavage site specificity within the transmembrane domain of its target proteins. Previous studies investigating the proteolytic processing of the γ-secretase substrate, p75^{NTR}, and its homolog NRH1 found that transmembrane cleavage of NRH1 was not sensitive to the γ-secretase inhibitor DAPT, suggesting that it is not processed by γ-secretase (Kanning *et al*. J. Neuroscience, 23: 5425). We have identified the zebrafish orthologues of the p75^{NTR} and NRH1 genes and developed *in-vivo* assays to assess the cleavage events of the p75^{NTR} and NRH1 proteins. Our results from these assays in zebrafish were consistent with previous studies. Inhibition of γ-secretase by DAPT treatment resulted in the accumulation of uncleaved p75^{NTR} substrate, while NRH1 cleavage remained unaffected. This suggests that p75^{NTR} is cleaved by γ-secretase and NRH1 is cleaved by a different γ-secretase-like activity. We have established a system in which we can now attempt to elucidate the structural basis for γ-secretase cleavage specificity. (200 words)

POS-THU-214

EFFECTS OF LOW VERSUS HIGH FREQUENCY VAGAL STIMULATION ON RESPIRATORY MOTOR OPUTPUT

Ajayi IE1,2, Mills PC 2, Balnave RJ 3 and Subramanian HH1+,

1*Asia-Pacific Centre for Neuromodulation, University of Queensland Centre for Clinical Research, The University of Queensland, Queensland 4029, Australia.*
2*School of Veterinary Science, The University of Queensland, Queensland 4343, Australia.*
3*School of Medical Sciences, The University of Sydney, Sydney 2001, Australia.*

Vagal Afferent feedback is critical in shaping respiratory motor output. Vagal sensory fibres have different structural and physiological transmission properties (for example, fibre diameters, myelination and stretch receptors with very different responses to lung and airway defamation). Vagal feedback to various respiratory brainstem centres are governed by the principles of habituation and de-sensitisation and thus could be dependent on frequency of vagal stimulation. We investigated changes in diaphragmatic firing pattern, the primary index of respiratory motor output, in response to electrical stimulation of the vagus at low (7Hz/5V) vs. high (50/Hz/5V) frequencies. The effects tracheal occlusion and hypercapnia in conjunction with vagal stimulation were also explored. Low frequency stimulation immediately inhibited inspiratory activity, however diaphragm activity rebounded when the stimulation was maintained for a prolonged time. On the other hand, high frequency vagal stimulation inhibited the diaphragm for the entire stimulation period. Tracheal occlusion induced an expiratory prolongation reflex, however inspiratory rhythm was restored even while occlusion was maintained. Hypercapneic drive (via ventilating the animal with 7% CO_2 in the inspired gas) immediately reversed the low-frequency vagal stimulation-induced inspiratory inhibition. However hypercapnia was not capable of over-riding high-frequency vagal stimulation-induced inspiratory inhibition. Low frequency vagal stimulation during hypercapneic breathing did not produce inspiratory inhibition. In all cases, hypercapnia induced changes in the frequency and amplitude of the diaphragm during post-stimulus recovery. These results are discussed with relevance to vagal mediated respiratory control, principles of habituation and de-sensitisation and how other respiratory interventions in combination with vagal stimulation influence breathing.