POSTER SESSION A

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POS-WED-001

EFFECT OF CHOLERA TOXIN ON COLONIC MIGRATING MOTOR COMPLEXES IN FEMALE MICE DEPENDS ON MUCOSAL 5-HT AND SEX STEROID HORMONES

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Purpose: Cholera toxin (CT) inhibits colonic migrating motor complexes (CMMCs) and constricts the colon of female C57Bl/6 mice, effects blocked by 5-HT_3 receptor antagonists. This occurs in estrus females, but not in proestrus females or males. We examined the effect of CT on CMMCs in female mice that selectively lack 5-HT in enterochromaffin cells (tryptophan hydroxylase 1 knockout; TPH1KO). Distributions of estrogen receptors (ER_α, ER_β) and aromatase in mouse colon were also characterised.

Methods: Spatiotemporal maps of CMMCs *in vitro* were constructed from video recordings. CT (1.25 µg/ml) was introduced to the colonic lumen of randomly selected female TPH1KO mice after control recordings with Krebs solution and later washed out. ER_α, ER_β, aromatase and a pan-neuronal marker (Hu) were localized immunohistochemically in whole mounts using relevant antibodies.

Results: In contrast to the wildtype, CT did not affect CMMCs in TPH1KO female mice ($p>0.05$, $n=7$), but still evoked a tonic constriction ($p<0.05$, $n=7$). ER_α and ER_β were expressed in nearly all myenteric neurons of female and male colon (female ER_α=87%, ER_β =95%; male ER_α=91%, ER_β =88%). Aromatase staining was also observed.

Conclusion: CT effects on CMMCs depend on mucosal 5-HT acting on 5-HT3 receptors, but the constriction caused by CT is independent of mucosal serotonin. Differences seen during the estrus cycle and in males may result from differences in sex steroid hormone levels, consistent with our finding that ER_α, ER_β and aromatase are expressed in colonic myenteric ganglia in mice of each sex. (241)

POS-WED-002

MECHANISMS UNDERLYING THE GENERATION OF COLONIC MIGRATING MOTOR COMPLEXES: DO THEY OCCUR IN AN EMPTY INTESTINE?

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Colonic migrating motor complexes (CMMCs) are cyclical contractions of the large intestine, recorded from isolated full length segments of colon. In previous studies, external stimuli have been applied to the intestine to enable recording CMMC activity. In this study, we investigated whether CMMCs occur in an empty intestine, without any external stimuli that are normally used to record this motor pattern. Spatio-temporal maps of colonic diameter were made from the isolated full length mouse colon at 36^{o} C. CMMCs occurred on average at 2.0 ± 0.2 CMMCs per minute in colons containing multiple faecal pellets (N=20) and they propagated along the full length colon at 2.16 ± 0.2 mm/sec. When these same preparations had expelled all content, CMMCs were absent in 9/20 preparations. In the remaining preparations where some content was present, CMMCs, occurred at 0.2 ± 0.03 CMMCs per minute and propagated only 43 ± 7.5% along the length of colon; at a significantly reduced velocity (0.75 ± 0.75 mm/sec; $P<0.05$). Stimulation of these empty preparations increased CMMC frequency in 4/11 preparations (mean 0.3 ± 0.04/min) and increased the extent of propagation to 64.9 ± 11.8 % and velocity 2.45 ± 0.6 mm/sec. In summary, we show that CMMCs occur rarely, or not at all, in isolated preparations of mouse colon devoid of intraluminal contents. The frequency, velocity and extent of propagation of CMMCs in vitro is dependent upon the presence of intraluminal content and/or the external stimuli that are normally used to record this motor pattern. (247 words)

POS-WED-003

BRAINSTEM LOCI INVOLVED IN LARYNGEAL ADDUCTION AND COORDINATION OF FICTIVE SWALLOWING *IN SITU*.

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Objective: Aspiration is a risk during swallowing, as the pharynx is a shared conduit for transit of ingested material and airflow. Entry into the airways is prevented by laryngeal adduction of the vocal folds. The Kölliker-Fuse nucleus (KF) provides pre-motor drive to laryngeal adductor motoneurons. We investigated the role of the: 1) KF, in laryngeal adduction during swallowing, and; 2) nucleus of the solitary tract (NTS), in the generation and coordination of swallows.

Methods: The phrenic, cervical vagus, and hypoglossal or iliohypogastric nerves were recorded in the *in situ*, perfused brainstem-spinal cord preparation of Sprague Dawley rat (P16-21). Fictive swallowing was elicited by oral injection of distilled water (0.2-0.6ml) before and after focal microinjection of GABA-A receptor agonists and antagonists into KF and NTS.

Results: Water stimulation evoked fast sequential swallows, observed as high amplitude bursts in cervical vagus nerve with tonic activity in between. Swallow motor patterning was intact following inhibition of KF, albeit with loss of the tonic activity and increased incidence of spontaneous swallows. Conversely, disinhibition of KF resulted in exaggerated tonic activity and delayed swallows. Swallowing was abolished after inhibition of the NTS. Disinhibition of NTS resulted in disordered responses to water stimulation.

Conclusions: Generation and coordination of swallowing is mainly subserved by the NTS. The KF provides additional background laryngeal adduction but is not the primary source of laryngeal adduction during swallows. This is of relevance to diseases featuring dysphagia including Alzheimer's disease and Rett Syndrome in which KF dysfunction has been implicated.

POS-WED-004

VAGALLY-MEDIATED GASTRIC RELAXATIONS ARE REDUCED IN *W/W^V* MICE LACKING INTRAMUSCULAR INTERSTITIAL CELLS OF CAJAL

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Intramuscular interstitial cells of Cajal (ICC-IM) form close associations with motor nerve terminals, vagal intramuscular arrays (IMAs) and smooth muscle cells within the gastric musculature. Previous studies examining the role of ICC-IM in neurotransmission have involved the non-discriminate electrical field stimulation of all nerves within the stomach wall. Here, the role of ICC-IM in the transduction of vagal input to the stomach was investigated using *W/W^V* mice, having reduced populations of gastric ICC-IM. Mechanical responses to vagal trunk stimulation (0.5ms pulses; 5-20Hz; 10s) were recorded from fundal and antral regions of wildtype and *W/W^V* stomachs before and after the cumulative addition of bethanechol, nitro-L-arginine (L-NA) and hexamethonium. In control conditions, vagal stimulation most commonly elicited wildtype stomach relaxation whilst the predominant response of *W/W^V* stomachs was contraction. Vagally mediated relaxations were compared following precontraction with bethanechol (3µM). In bethanechol, relaxations to vagal stimulation (10Hz) were of smaller amplitude in *W/W^V* fundus and antrum compared to wildtype counterparts. L-NA (100µM) reduced fundal relaxations to 10Hz vagal stimulation (from17.3±3.5mN to 6.6±2.3mN in wildtype ($p=0.001$); and from 6.1±1.4 to 2.8±1.3mN in *W/W^V* ($p=0.04$)). In L-NA, residual relaxations of *W/W^V* fundus to vagal stimulation were abolished by hexamethonium (500µM), whereas, in wildtype fundus small amplitude relaxations persisted and were abolished by cutting vagal trunks. These data support the hypothesis that ICC- IM are important for the transduction of vagally-mediated gastric relaxations. Lack of hex-resistant relaxations in *W/W^V* provides a functional correlate for the morphological observation that IMAs fail to ramify into gastric muscles lacking ICC-IM. (250 words)

POS-WED-005

CHARACTERISATION OF PELVIC AUTONOMIC NEURONS FOLLOWING AXOTOMY REVEALS NEURONAL POPULATIONS WITH DISTINCT GROWTH PROFILES

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Pelvic ganglia (PG) comprise sympathetic and parasympathetic neurons that innervate the urogenital organs. These axons are commonly injured in surgical procedures yet their regenerative capacity is poorly understood. Activating transcription factor 3 (ATF-3) and c-Jun are upregulated in axotomised neurons and considered essential for regeneration. Using these two injury markers, we sought to determine the regenerative activity of bladder motor neurons. Experiments were conducted on adult male Wistar rats (min 4/group); surgery was performed under isoflurane anaesthesia. All results referred to below achieved statistical significance ($P<0.05$). First, retrograde tracers were microinjected into the bladder to label bladder-projecting PG neurons; different tracer dyes distinguished neurons innervating each side. One week later, the bladder was denervated unilaterally (accessory nerve transection), and PG harvested after a further 1 or 3 weeks then processed for immunofluorescence. About one-third of sympathetic and parasympathetic bladder neurons expressed ATF-3 after injury in the ipsilateral (injured) but not contralateral (uninjured) ganglion, however c-Jun was upregulated in both PG. This study has shown that injury markers are upregulated in a subpopulation of bladder projecting neurons ipsilateral to injury, however their restriction to a minority of neurons suggests that neurons differ in their regenerative capacity. Previous studies on cavernosal projections have demonstrated c-Jun expression in uninjured, sprouting PG neurons, so our results suggest that some uninjured bladder efferents undergo axonal sprouting to re-establish neural control of denervated tissue on the contralateral side. Together these data improve our understanding of the responses of pelvic autonomic circuits to injury.

(247 words)

POS-WED-006

MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY BY LOW-FREQUENCY PHYSIOLOGICAL ACTIVATION OF THE VESTIBULAR SACCULE IN HUMANS

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Purpose: The utricle and saccule detect linear displacement of the head in the horizontal and vertical planes, respectively. We previously showed that sinusoidal physiological activation of the utricle in upright humans causes a pronounced modulation (X-axis 32±3 and Y-axis 29±3 %) of muscle sympathetic nerve activity (MSNA), suggesting a significant role for the utricle in the control of blood pressure during postural challenges (1). Here we tested the hypothesis that the saccule can also play a role in blood pressure regulation by modulating lower limb MSNA.

Methods: MSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 9 subjects. Subjects lay supine on a motorised platform and slow sinusoidal linear accelerations-decelerations (~4 mG) were applied rostro-caudally (X direction; predominantly saccule stimulation) and medio-laterally (Y direction; predominantly utricle stimulation) at 0.08 Hz; heart rate (ECG), respiration, blood pressure and skin blood flow were also recorded. Results: Cross-correlation analysis revealed sinusoidal modulation of MSNA in most (7/9) subjects. One had no modulation, 2 were modulated in only one direction. The saccular modulation of MSNA (X = 27±5%) was similar to the utricular modulation (Y = 32±5%) when supine; both were significantly lower than the coupling between MSNA and ECG (X=82±2%, Y=85±2%).

Conclusion: We conclude that both the saccule and utricle modulate MSNA, although significantly ($P<0.05$) less than the magnitude of cardiac modulation of MSNA. The results suggest that both the saccule and utricular vestibular organs play a role in the control of blood pressure regulation during postural challenges. *(249 words)*

1) Hammam E, Kwok K & Macefield VG (2013) *Exp Brain Res* 230: 137–142

POS-WED-007

ESTROGENIC MODULATION OF TRKA SIGNALLING IN PC12 CELLS.

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Estrogens have widespread physiological effects including neuroprotective actions within the brain. The mechanisms underlying estrogenic neuroprotection are unknown but may involve modulation of neurotrophic signaling. This study has employed PC12 cells to investigate a possible interaction between 17-β-estradiol and the nerve growth factor (NGF) receptor TrkA. Single-molecule tracking of an antibody-labeled TrkA receptor on the plasma membrane of a live PC12 cell demonstrated that the estrogen increased the periods of receptor immobility ($p<0.05$) indicating enhanced receptor signalling. Consistent with this suggestion 17-β-estradiol also significantly increased NGF-mediated tyrosine phosphorylation of the TrkA receptor ($p<0.05$). In contrast, estrogenic stimulation did not increase the phosphorylation of ERK1/2, a downstream signalling molecule of TrkA. Indeed high concentrations of 17-β-estradiol (>1nM) significantly suppressed NGF-mediated ERK1/2 activation. Chronic exposure (2-6 days) of PC12 cells to 17-β-estradiol resulted in an NGF-like differentiation response, with a cessation of cell division and an outgrowth of neurites. Interestingly, this estrogenic response was reproduced by a GPR30-selective agonist, suggesting the involvement of a membrane localized estrogen receptor. These data provide evidence that estrogens modulate both acute and chronic TrkA receptor signalling in the PC12 cell model system. Such an interaction may provide a mechanism by which estrogens are neuroprotective through the enhancement of the action of neurotrophins such as NGF.
(209 words)

POS-WED-008

NITRERGIC NEUROTRANSMISSION IN THE PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS MODULATES AUTONOMIC RESPONSES EVOKED BY ACUTE RESTRAINT IN RATS

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Objective: The present work studied the possible involvement of PVN nitrergic neurotransmission in the mediation of restraint stress-induced autonomic changes. Methods: Guide cannulas were implanted into the PVN of rats. A catheter was introduced into femoral artery for blood pressure and heart rate recordings. We submitted rats to acute restraint and studied the effect of the microinjection of the selective neuronal NO synthase (nNOS) inhibitor N^{w}-Propyl-L-arginine (N-Propyl, 0.4/100nL), NO scavenger carboxy-PTIO (C-PTIO, 1nmol/100nL) into the PVN on increases in blood pressure, increase in heart rate and decreases in tail temperature induced by acute restraint stress. Results: The N-Propyl or C-PTIO pretreatment of the PVN significantly reduced the restraint-evoked increase in MAP response (N-Propyl: $F_{1,700}$= 253.1, P<0.0001, n=6; C-PTIO: F1,700= 309.4, P<0.0001, n=6) and HR (N-Propyl: $F_{1,700}$=7.8, P>0.05, n=6; C-PTIO: $F_{1,700}$=81.3,P<0.0001, n=6) response, when compared to animals treated artificial cerebrospinal fluid (ACSF, vehicle, 100nL). Moreover, N-Propyl or C-PTIO treatment of the PVN significantly reduced the restraint-evoked decrease in tail skin temperature (N-Propyl: $F_{1, 90}$= 13.4, P<0.05, n=6; C-PTIO: $F_{1, 90}$= 15.3, P<0.05, n=6), when compared with ACSF-treated animals. Conclusion: These results show that local PVN nitrergic neurotransmission participate in the neural pathway which is involved with autonomic responses observed during acute restraint stress. Financial Support: The State of São Paulo Research Foundation (FAPESP 2009/05308-8; 2012/09425-1). (214 words)

POS-WED-009

TREATMENT WITH ANTI-CANCER DRUG OXALIPLATIN AFFECTS NEUROMUSCULAR TRANSMISSION IN THE MURINE DISTAL COLON

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Oxaliplatin is one of the first-line therapies to treat colorectal cancer, however its use is associated with peripheral neurotoxicity and severe side-effects. Damage to enteric neurons may underlie the gastrointestinal side-effects, which include diarrhoea, constipation, nausea and vomiting. We have previously shown that following a 14 day oxaliplatin-treatment the number of myenteric neurons in the murine distal colon decreased compared to sham-treatment. However, the proportion of nitric oxide (NO) synthesising neurons increased and their cell bodies were larger. This correlated with a decrease in colonic motility *in vitro* (Wafai et al., 2013). Nitrergic neurons provide inhibitory inputs to the muscles and other neurons. In the current study we investigated whether morphological changes in these neurons directly affected their functions. Intracellular recordings of junction potentials induced by stimulating motor neurons were made from circular muscle cells in preparations of murine distal colon following 14-days of *in vivo* treatment with either oxaliplatin or saline. The amplitude of nitrergic-inhibitory junction potentials was significantly larger in cells from oxaliplatin-treated versus sham-treated mice (-7.3±1.0mV vs -5.4±0.6mV, respectively, $P<0.05$, $n=4$). The amplitudes of purinergic-inhibitory junction potentials and cholinergic-excitatory junction potentials were not significantly different ($n=4$). The resting membrane potential and input resistance did not differ ($n=4$). Our results show only NO-mediated junction potentials in colonic circular muscle are affected by oxaliplatin-treatment. To investigate whether oxidative stress may be a cause for this response, immunohistochemical anaylsis for protein nitrotyrosine formation and labelling of superoxide formation by the mitochondria with Mito-SOX will be used. (WORD LENGTH: 248)

Wafai, L., Taher, M., Jovanovska, V., Bornstein, J.C., Dass, C.R., and Nurgali, K. (2013). Effects of oxaliplatin on mouse myenteric neurons and colonic motility. *Front. Neurosci.* 7**,** 30.

POS-WED-010

SPONTANEOUSLY HYPERTENSIVE RATS HAVE MORE OREXIN NEURONS IN THE MEDIAL HYPOTHALAMUS THAN NORMOTENSIVE RATS.

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The neuropeptide orexin makes a significant contribution to the regulation of blood pressure as part of its role in the maintenance of wakefulness and the control of arousal. This is consistent with the location of the orexin neurons in the dorsal hypothalamus and the presence of orexin terminals in the rostral ventrolateral medulla (RVLM) and the intermediolateral (IML) column of the thoracic cord. Recent work has shown that pharmacological blockade of orexin receptors reduces blood pressure in spontaneously hypertensive rats (SHR). It is not clear why the orexin system is upregulated in these animals, but one possibility is that SHR have more orexin neurons and/or a greater density of orexin terminals in the RVLM and IML. SHR male rats (n=12) and normotensive Wistar Kyoto (WKY, n=11) and Wistar (n=5) male rats were euthanized, perfused and their brains and spinal cord sectioned and immunolabelled for orexin A. Labelled neurons were plotted and counted in the six densest hemisections (120 µm apart) of each brain. The results show a significantly higher number of orexin neurons in the medial hypothalamus (medial to fornix) in hypertensive SHRs when compared to normotensive WKYs and Wistars (125±5 vs 105±5 and 104±6 per hemisection, respectively, $p<0.03$). Orexin terminals in the RVLM and IML were also significantly denser in SHR than in WKY ($p<0.04$). These experiments support the idea that the orexinergic system is upregulated in SHR, and suggest that this may be due in part to a greater number of orexin neurons in the medial hypothalamus. (249 words).

POS-WED-011

CENTRAL ADMINISTRATION OF PALMITIC ACID AND ARACHIDONIC ACID REGULATE PERIPHERAL ENERGY HOMEOSTASIS BY DECREASING CENTRAL LEPTIN SENSITIVITY IN MICE

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Purpose: Leptin acts on the central nervous system to induce negative energy balance. High fat diets enriched with saturated fatty acids (SFA) and n-6 polyunsaturated fatty acids (n-6 PUFA) have been reported to contribute to positive energy balance and promote obesity. This study examined central leptin sensitivity as well as glucose and lipid metabolism in liver in response to central injection of either SFA palmitic acid (PA) or n-6 PUFA arachidonic acid (ARA).

Methods: Overnight fasted male C57BL/6J mice were administered an intracerebroventricular (icv) injection of PA, ARA or vehicle, followed by icv injection of leptin or saline; after which food intake was measured. Three days later, the above sequence of icv injections was repeated. Liver was collected 1 hour after the second leptin injection. The gene expressions of key enzymes of glucose (PEPCK, G6Pase, and GLUT2) and lipid (FAS, SCD1, and HMG-CoA reductase) metabolism were determined by RT-PCR.

Results: In vehicle pre-treatment group, central leptin administration significantly suppressed food intake at 24 hours (52.72%, $p<0.001$) compared to the saline control. However, in PA and ARA pre-treatment groups, leptin did not significantly suppress food intake. With vehicle pre-treatment, PEPCK ($p<0.001$), G6Pase ($p<0.01$), GLUT2 ($p<0.05$), FAS ($p<0.05$), SCD1 ($p<0.05$), and HMG-CoA reductase ($p<0.05$) significantly decreased in response to central leptin, while no change was observed in fatty acid pre-treatment groups.

Conclusion: This study suggests that central administration of SFA and n-6 PUFA induced central leptin resistance, and attenuated the central leptin in regulation of gluconeogenesis, lipogenesis and beta-oxidation in liver.

POS-WED-012

THE OPIOID AGONIST BETA-CASOMORPHIN PRODUCES DYSMOTILITY IN THE RAT LARGE INTESTINE.

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Opioids act on receptors on enteric neurons to inhibit acetylcholine-induced muscle contraction. The casein-derived peptide beta-casomorphin 5 (opioid agonist) is reported to slow gastro-intestinal (GIT) transit in the rat and to block the peristaltic reflex in isolated guinea pig colon. To determine the minimum tissue preparation necessary to screen for similar substances that affect GIT motility we investigated how beta-casomorphin 5 affects the muscle contraction profile in rat distal colon segment compared with the rat whole large intestine. Distal colon segments (four per animal) were placed in oxygenated tissue baths of Kreb's buffer at 37 °C under 1 g of tension longitudinally. Whole large intestine was treated similarly and tensioned transversely at four evenly spaced sites from which propagating contractions were measured. Differences in tension and frequency were measured relative to spontaneous contractile activity. Following a 2 h equilibration, beta-casomorphin 5 (0.2 - 20 µM) was applied for 15 min (n = 7-8). In the distal colon, beta-casomorphin 5 increased the tension and frequency of contractions in a concentration-dependent manner. The increased motility produced by 20 µM beta-casomorphin 5 was inhibited by the naloxone opioid antagonist. In the large intestine 20 µM beta-casomorphin 5 disrupted the synchrony of propagating contractions. Our results indicate that while short distal colon segments were pharmacologically informative, this only showed part of the picture. The large intestine preparation had a more intact enteric nervous system and the disruption to propagating contractions suggests that this is how beta-casomorphin 5 slows gastro-intestinal transit. (246 words)

POS-WED-013

DISTRIBUTION OF OREXIN RECEPTORS IN CENTRAL AUTONOMIC CENTRES REVEALED BY TRIPLE IMMUNOFLUORESCENCE

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Orexins (hypocretins) are excitatory neuropeptides synthesized in the central nervous system exclusively by neurons of the lateral hypothalamus. Orexin-containing neurons have widespread projections and have been implicated in complex physiological functions including feeding behaviour, sleep states, neuroendocrine function, and autonomic control. Two orexin receptors (OX1R and OX2R) have been identified. Previous studies analysing mRNA expression have indicated distinct and differential expression patterns of OX1R and OX2R throughout the central nervous system. Here we demonstrate a distinct pattern of orexin receptor expression within autonomic centres through the use of triple immunofluorescent labelling. Wistar rats were euthanized and perfused and sections of their brains and spinal cords processed for triple immunofluorescence of OX1R, OX2R and either orexin-A, tyrosine hydroxylase (TH) or Choline acetyl transferase (ChAT). The results show that 63±3% of orexin neurons expressed both OX1R and OX2R, 20±2% only OX1R and 3±1% only OX2R (n=3). However presympathetic neurons in the C1 and A5 group of the caudal pons and rostral medulla expressed largely only OX1R (81±7% of TH-labelled C1 neurons, 92±2% of TH-labelled A5 neurons, n=4), and never OX2R. Furthermore, sympathetic preganglionic neurons in the intermediolateral column of the spinal cord were also found to express mainly OX1R (89±3% of ChAT-labelled neurons, n=2), with a small proportion expressing both OX1R and OX2R (3±1%). This suggests that the action of orexin on autonomic centres is potentially largely mediated via OX1R, and could thus prove a potential pharmacological target for the amelioration of stress and anxiety induced hypertension. (245 words).

POS-WED-014

THE EFFECT OF GESTATIONAL HIGH FAT DIET ON CARDIOVASCULAR AUTONOMIC RESPONSES TO DEHYDRATION

Dissanayake H.U.W. and Polson J.W.
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A maternal high fat diet during gestation (MHFD) programmes for metabolic disease, including hypertension risk, in the offspring. A previous study reported high salt diet increased blood pressure (BP), associated with increase sympathetic activity in MHFD, but not control rats (1). In this study we tested the effect of another osmotic stimulus, dehydration, on cardiovascular autonomic responses. Rat dams were placed on a high fat diet (59% fat) for one month before mating until parturition. Control dams were fed a standard chow (5% fat). At 6 months age, the offspring were implanted with telemetry BP transmitters. BP and derived spontaneous baroreflex sensitivity (sBRS), heart rate variability (HRV) and BP variability (BPV) were measured over a 9 day protocol, comprising 3 days baseline, 3 days without water and 3 days recovery. MHFD offspring had higher systolic BP than controls throughout the protocol (baseline BP: MHFD, 130±10 vs control, 115±6 mmHg, n=4, $P<0.01$). This was associated with increased low frequency:high frequency HRV (0.58±0.09 vs 0.34±0.02, $P<0.01$) and sBRS (2.4±0.4 vs 1.5±0.3, $P<0.01$) in females. Dehydration evoked an increase in BP, accompanied by an increase in low frequency HRV (37±7%, $P<0.05$) and BPV (56±16%, $P<0.05$). In the MHFD female, there was a trend towards an increased sBRS (35±15%, $P=0.06$). Following rehydration, BP fell ~10 mmHg below baseline levels and remained reduced. These results showed that MHFD elicited hypertension associated with altered autonomic function. However, there were no significant differences in the cardiovascular and autonomic responses to dehydration in these rats.

(1) Rudy O. et al., PLOS ONE 6: article number e25250, 2011.
(247 words)

POS-WED-015

GLYCEROL STIMULATES MUCOSAL AFFERENTS AFFECTING DISTENSION-INDUCED PERISTALSIS IN THE RAT COLON

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Intestinal propulsion is mostly due to aboral propagation of rings of circular muscle contractions (peristaltic contractions) controlled by enteric neural circuits. However, the role of intrinsic or extrinsic afferent mucosal nerve endings has not been established. *Ex vivo* isolated rat colon generates neurally-dependent peristaltic contractions initiated by luminal fluid distension (1). We investigated whether glycerol, used clinically as a laxative, affects distension-induced peristaltic contractions via nerve endings located in the mucosa. **Methods**: Isolated whole rat colon was placed in an organ bath containing oxygenated Krebs solution (37°C) and motor activity was studied using spatio-temporal maps of changes in diameter constructed from video recordings. **Results:** Continuous infusion of Krebs at the oral end of the colon (1.5 ml/min) triggered regular neural peristaltic contractions that emptied intraluminal content. Luminal infusion of lidocaine (100 µM) did not block peristaltic contractions, although decreased their frequency (0.4±0.02 vs. 0.5±0.03 cpm in controls; $P<0.01$) and extent of propagation (70±10 vs. 80±4 % of total length; $P<0.03$), while increasing maximal diameter prior to contraction (6.1±0.2 to 6.6±0.2 mm; $P<0.03$). Intraluminal glycerol (30%) increased the frequency of peristaltic contractions from 0.4±0.03 to 0.9±0.1 cpm ($P<0.03$), each ejecting less fluid volume compared to control (2.5±0.3 to 1.1±0.6 ml; $P<0.003$). This effect of glycerol was reversibly blocked by subsequent intraluminal application of lidocaine. **Conclusions:** Glycerol appears to reduce the threshold for distension-evoked peristaltic contractions by stimulating mucosal afferent nerve endings, which can be selectively blocked by luminal infusion of lidocaine. (250 words)

1. Costa *et al*. 2013. *Am. J. Physiol*. In press.

POS-WED-016

OREXIN/HYPOCRETIN NEUROPEPTIDES IN THE DEVELOPING PIGLET HYPOTHALAMUS AFTER INCREASING DURATION OF INTERMITTENT HYPERCAPNIC HYPOXIA.

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Orexin (OxA and OxB) and its receptors are shown to be involved in maintenance of sleep and arousal, and to be regulated by various environmental stimuli. To mimic the pathological mechanism of obstructive sleep apnoea (OSA), and the two associated common risk factors of sudden infant death syndrome (SIDS), bed-sharing and prone sleeping, piglet models of intermittent hypercapnic hypoxia (IHH; 7%O_2/8%CO_2) during early post-natal period, were developed. Immunoreactivity of OxA and OxB was investigated in the piglet hypothalamic regions of the dorsomedial nucleus (DMN), perifornical area (PeF) and lateral hypothalamic area (LHA). Comparing the three IHH exposure durations 1-Day (1D-IHH) (n=7), 2-Days (2D-IHH) (n=7) and 4-Days (4D-IHH) (n=6) to controls (exposed to normal air, n=7), we found that IHH induced an overall decrease in orexin expression. Specifically, after 1D-IHH, only OxB was decreased and was seen in the DMN and PeF ($p<0.01$). After 2D-IHH, OxA was decreased in LHA ($p=0.02$), while OxB was decreased in all 3 regions [DMN ($p<0.001$), PeF ($p<0.01$), LHA ($p=0.03$)]. After 4D-IHH, the decreased OxB in PeF ($p<0.01$) was maintained. These findings indicate that repetitive IHH exposure induces greater changes than an acute 1-day exposure, with changes levelling out after 4 days (possibly habituation), and suggest a role of orexin in the pathogenesis of OSA and SIDS. (211 words).

POS-WED-017

INTER-INDIVIDUAL DIFFERENCES IN BLOOD PRESSURE RESPONSES TO STRESSORS: MENTAL VERSUS PHYSICAL TASKS

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Purpose: Chronic stress has been linked with high blood pressure (BP) status. Evidence suggests that inappropriately large BP increases during mental stress may be involved in the development of hypertension. However, what causes elevated cardiovascular reactivity to stress in some individuals but not others remains unclear. The aim is to determine what drives the BP responses to mental and physical stress in healthy young individuals. **Methods:** Muscle sympathetic nerve activity (MSNA) (*via* microneurography), BP and heart rate (HR) 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). Mean responses during each 2-min stressor test were compared to resting values for the 2-min period immediately prior to the task. Individuals who exhibited large increases in systolic BP (≥10 mmHg) were defined as 'responders' while those who exhibited increases <10 mmHg were defined as 'non-responders'. **Results:** For the cold pressor test, responders (mean ΔBP = 21 mmHg) exhibited significantly greater increases in MSNA than non-responders (mean ΔBP = -5 mmHg) (P=0.032). For the mental arithmetic task, the differences in MSNA and HR between responders (mean ΔBP = 19 mmHg) and non-responders (mean ΔBP = 3 mmHg) did not reach statistical significance, despite a trend for greater increases in HR in responders. **Conclusions:** The large BP increases in responders during physical tasks are driven by MSNA, while our data suggest that HR may drive the increase in BP during mental tasks. (247 words).

POS-WED-018

AN ACTIVE GRE SITE IN CRH PROMOTER CONTRIBUTES TO THE SUSCEPTIBILITY TO STRESS IN CHINESE TREE SHREW

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Tree shrew (*Tupaia belangeri*), more closely related to primates than rodents, has been regarded as a promising model to study on stress-related disorders. But the mechanism underlying the susceptibility to stress still remains unknown.

In our study, the sequence of full length CRH gene of Chinese tree shrew was resolved by genome walking, which showed a high homology of 83% with human CRH gene in coding sequence region and 73% identity in full length. Compared with human CRH peptide, a single amino acid change was found in tree shrew CRH peptide. Similar to human CRH, synthetic tree shrew CRH peptide was capable to induce MAPK pathway activation, POMC transcription and ACTH release, but did not show obvious difference in the above aspects. In the promoter activity analysis, an active GRE site in CRH gene promoter region was identified as a key element responsible for higher promoter activity in tree shrew than that of human under the basal condition or treatment with forskolin and/or dexamethasone. Our results highlight a critical role of the active GRE site through binding with Glucocorticoid receptor in regulating CRH mRNA expression, which contributes to higher promoter activity and susceptibility to stress in tree shrew than in human. (201 words).

POS-WED-019

MONOAMINE AXONS PREFERENTIALLY INNERVATE OESOPHAGEAL MOTOR NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS

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Neurons in the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus pars compact (NAc) innervate the subdiaphragmatic oesophagus in rodents. However, little is known about the central circuitry that controls the activity of these neurons. We assessed the monoamine innervation of these two groups of neurons with immunohistochemistry, conventional retrograde tracing and anterograde tracing using lentiviral vectors that drive GFP expression in either catecholamine or 5-HT neurons. Sections of medulla from rats with cholera toxin B subunit (CTB) injected into the subdiaphragmatic oesophagus were immunoperoxidase stained for CTB (brown) and either tyrosine hydroxylase (TH), phenylethanolamine-N-methyltransferase (PNMT) or 5-HT (black). Sections from rats with brainstem injections of lentiviral vectors that drive expression of GFP exclusively in either catecholamine neurons or serotonin neurons were immunostained for GFP (black) and choline acetyltransferase (ChAT; brown). In the DMV, many CTB-positive esophageal motor neurons received close appositions from PNMT-, TH- and 5-HT-immunoreactive axons. However, all three types of axons very rarely apposed CTB-labelled motor neurons in NAc. In rats with lentiviral-induced selective GFP expression in C1, C3 or midline 5-HT neurons, GFP-immunoreactive axons were found in close apposition to ChAT-positive motor neurons in DMV but not in NAc. This marked difference in innervation indicates that monoamine axons of brainstem origin are likely to be important for controlling the activity of esophageal motor neurons in DMV but not those in NAc. This conclusion is consistent with control of oesophageal smooth muscle by DMV neurons and of oesophageal striated muscle by NAc neurons.

POS-WED-020

SENSORY RE-INNERVATION OF THE POST-PARTUM RAT UTERUS

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Sympathetic, parasympathetic and sensory nerves have almost completely disappeared from the pregnant rat uterus at term. Uterine re-innervation post-partum is not well characterized. We assessed sensory re-innervation of the rat uterus in the first 2 weeks post-partum using whole mount preparations. We fixed rat uterine horns on post-partum (P) days P1, P3, P5, P7, P10 and P14 (n=4 at each time-point). The whole mounts were stained for calcitonin gene related peptide (CGRP) using peroxidase immunohistochemistry and then resin-embedded. At P1, there were few CGRP-immunoreactive axons, mostly at the mesometrium where a few blood vessels were lightly innervated. CGRP-positive axons increased at P3 and P5 although muscle innervation remained sparse. Many growth cones were present at both time points. Innervation was denser at P7, with slightly more muscle innervation and rare CGRP-IR axons deep in the endometrium. There were CGRP-immunoreactive axons in the linea uteri at the ovarian end in half the rats. At P10, the pattern of CGRP innervation was similar to P7. There were more CGRP-immunoreactive axons in all regions but innervation of the middle and cervical linea uteri was absent in 3 of 4 rats. CGRP innervation was heaviest at P14, with blood vessels significantly innervated. Nevertheless, the density of CGRP innervation in all uterine layers was still less at P14 than at oestrus. These results show that sensory innervation of the uterus has begun to return at P1. Uterine CGRP innervation increases to P14 but innervation density is still lower than in the non-pregnant state. (248 words)

POS-WED-021

BEHAVIOURAL PROFILE OF RELAXIN-3/RXFP3 DEFICIENT MICE IN THE METHAMPHETAMINE WITHDRAWAL MODEL OF MONOAMINE DEPLETION AND DEPRESSION

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In rodents and humans, brain monoamine signalling can be reduced after withdrawal from chronic methamphetamine administration, which can lead to increased anxiety, anhedonia, bodyweight loss and other depressive-like symptoms. The neuropeptide relaxin-3 and its cognate receptor, RXFP3, represent a newly identified 'ascending arousal system', which shares neuroanatomical and functional similarities with the serotonin/dorsal raphe and noradrenaline/locus coeruleus systems. Serotonin depletion increases relaxin-3 expression in rodents, which we hypothesised might partially protect/compensate against the depressive-like symptoms induced by chronic methamphetamine withdrawal (CMW). Therefore, female relaxin-3 and RXFP3 knockout (KO) mice and their wildtype (WT) littermates were injected with saline or 6 mg/kg (i.p) methamphetamine (*n*=16-19/group) once daily for 10 days, and behaviour was assessed during the subsequent 3 week withdrawal period. Relaxin-3/RXFP3 KO mice displayed similar behavioural responses to CMW as WT controls ($p>0.05$) in paradigms which measured anxiety (light/dark box and elevated plus maze), anhedonia (saccharine preference) and depressive-like behaviour (Porsolt swim); although CMW only induced minimal behavioural changes in WT mice. Interestingly, however, both relaxin-3 and RXFP3 KO mice lost significantly more bodyweight than WT littermates during CMW ($p<0.05$). These data provide further evidence that relaxin-3/RXFP3 signalling can modulate hypothalamic and limbic circuits involved in feeding and motivation, which are also targeted by monoamine systems and are aberrant in depression. Studies are ongoing to determine the level of monoamine depletion achieved (by HPLC analysis), and phenotypic differences will be further explored by qPCR analysis of gene expression in key brain regions. (242 words).

POS-WED-022

THE EFFECT OF ORTHOGNAL SHEARING OF THE VESTIBULAR UTRICLE IN HUMANS ON MUSCLE SYMPATHETIC NERVE ACTIVITY

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Purpose: We have repeatedly shown that sinusoidal galvanic vestibular stimulation and physiological activation of the vestibular apparatus causes pronounced modulation of sympathetic nerve activity to both muscle and skin at a wide range of frequencies. In this study we sought to investigate the force-response relationship of the utricular hair cells on the vestibulosympathetic reflex.

Methods: Oligounitary MSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 13 subjects. Subjects were seated vertically on a motorised platform and sinusoidal accelerations-decelerations (100 cycles) were applied in the antero-posterior direction at a range of accelerations (1.25, 2.5, 5, 10, 20 & 30 mG), introduced in a quasi-random fashion, at a constant frequency of 0.2 Hz.

Results: Cross-correlation analysis revealed a marked sinusoidal modulation of MSNA, but like our previous studies conducted at >0.2Hz only one peak was generated. The magnitude of this modulation was 15.5±1.2, 18.9±1.9, 19.7±1.7, 17.2±1.4, 22.04±2.4 and 25.3±3.7 %, respectively. This was significantly lower than the coupling between MSNA and ECG (85.0±1.9, 86.6±1.7, 85.8±2.8, 83.7±3.4, 85.71±2.9, 84.6±2.5 & 84.5±3.3 %). With the exception of 10 mG, vestibular modulation of MSNA increased gradually with the incremental increase of the acceleration, although this did not reach statistical significance.

Conclusions: We conclude that physiological activation of the vestibular utricle causes a pronounced modulation of MSNA, the magnitude of which tended to increase with an increase in acceleration. Importantly, modulation of MSNA was apparent at accelerations (<10 mG) that could not be detected.

POS-WED-023

ACUTE OLANZAPINE TREATMENT BLOCKS THE HISTAMINE H1 RECEPTORS LEADING TO ACTIVATED AMPK SIGNALLING IN THE HYPOTHALAMIC ARCUATE NUCLEUS AND BRAINSTEM DORSAL VAGAL COMPLEX

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Olanzapine treatment is associated with severe obesity. The hypothalamic histamine H1 receptor (H1R) and AMP-activated protein kinase (AMPK) signalling have been identified as important contributors to olanzapine-induced weight gain. The brainstem dorsal vagal complex (DVC) is significantly involved in olanzapine-induced obesity. Aim: To examine the acute effect of olanzapine on the hypothalamic and DVC H1R-AMPK signalling using the parenteral rapid-acting intramuscular formulation of olanzapine. Methods: Rats were intramuscularly injected with olanzapine or water for 1, 9 and 18 injections (1 mg/kg, t.i.d., n=6/group). Cumulative food intake and weight gain were measured. The hypothalamic and DVC H1R, AMPK and phosphor-AMPK expression were examined using western blot. Results: Olanzapine started to cause hyperphagia after 9 injections ($p \leq 0.045$). After a single injection of olanzapine, the pAMPK/AMPK ratio, an indicator of AMPK activity, significantly increased in the DVC (20%, $p = 0.007$), and tended to increase in the hypothalamic arcuate nucleus (Arc) (16%, $p = 0.07$). After 9 injections, olanzapine significantly increased the pAMPK/AMPK ratio in the Arc (17%, $p = 0.003$) and DVC (10%, $p = 0.023$). The Arc and DVC pAMPK/AMPK ratio positively correlated with feeding efficiency and weight gain ($r \geq 0.643$, $p \leq 0.045$). Although the H1R expression in the Arc and DVC was not altered by olanzapine, it correlated with pAMPK expression ($r \geq 0.544$, $p \leq 0.068$). However, AMPK was not significantly activated after 18 injections in the two regions. Conclusion: Both the Arc and DVC H1R-AMPK signalling may play a role in mediating olanzapine-induced hyperphagia.

POS-WED-024

CHARACTERIZATION OF THE PRIMARY SPINAL AFFERENT INNERVATION OF THE MOUSE UTERUS: A COMBINED RETROGRADE AND IMMUNOHISTOCHEMISTRY STUDY.

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The primary afferent innervation of the uterus is poorly understood. Our aim was to identify the location and characteristics of sensory neurons in dorsal root ganglia that innervate the mouse uterus and identify the different morphological types of CGRP immunoreactive nerve endings. Using retrograde tracing, Di-i injections into the uterine wall revealed a biomodal distribution of DRG cell bodies with peak labelling occurring at two peaks, one from T13-L3 and a second smaller peak from L6-S1. The mean cross sectional area of labelled cells was 463 μm^2 +/- SEM. A significantly greater ($P<0.05$) proportion of labelled small neurons (<300 μm^2) was found in the sacral spinal cord (S2) compared with the peak labelling at the lumbar (L2) region. Immunohistochemisty of whole mount and sections of uterus revealed substantial innervation of the mouse uterus by CGRP-positive nerve fibres in the border between the circular and longitudinal muscle (N=4) The nerve endings could be resolved as free nerve fibres, that were further subclassified as either single or branching-type that aligned preferentially in the axis of either the circular or longitudinal smooth muscle. Complex endings were typically associated with mesenteric vessels. We have identified that the cell bodies of primary afferent neurons that innervate the mouse uterus lie primarily at L2 and S2 spinal levels, with peak CGRP immunoreactivity in the muscuaris externa. Multiple types of peptidergic nerve ending innervate the smooth muscle layers. This information will form the basis of future investigation into the mechanisms underlying nociception in the mammalian uterus.

POS-WED-025

TRACING SYNAPTIC INPUTS TO ENTERIC VISCEROFUGAL NEURONS

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Introduction: Viscerofugal neurons are enteric interneurons that project axons out of the gut to sympathetic neurons, forming reflex arcs that modulate gut motility. They are myenteric S-neurons which receive fast EPSPs from other enteric neurons. However, the sources of these inputs are unknown. **Methods:** Flat sheet preparations of guinea-pig colon, with submucosa removed and DiI applied to mesenteric nerves, were placed in organotypic culture. After 2-4 days, targeted intracellular recordings were made from longitudinal muscle-myenteric plexus preparations. **Results:** Thirty-one viscerofugal neurons were impaled, confirmed by co-localization of DiI and intracellularly injected carboxyfluorescein. Passive membrane properties were similar to those previously reported (RMP= -47.7±3.9mV, R_{in}=120MΩ, τ=7.7ms, N=8). Eleven of 20 neurons fired 1-3 action potentials at the onset of depolarizing current pulses;7/20 neurons fired repetitively; 2 were inexcitable. Spontaneous action potential firing occurred in 7/31 neurons. Circumferential electrical stimulation 0.5mm from impaled neurons evoked large amplitude EPSPs (9.8±5.3mV, 17/22 neurons) which were often suprathreshold (7/17). Ongoing, smaller amplitude EPSPs occurred in most neurons (22/26, 6.2±2.6mV). The nicotinic agonist DMPP was focally pressure ejected onto myenteric ganglia (10μM, 8 cells, N=4). DMPP directly applied to viscerofugal neurons evoked large depolarizations. In most ganglia (119/152) DMPP did not evoke detectable synaptic potentials. However, fast EPSPs were evoked at 33/152 ganglia, located up to 1.46 mm oral, 1.55mm circumferential, and 1.52mm anal to viscerofugal neurons. **Conclusion:** Myenteric ascending, descending and circumferential pathways contribute to excitation of viscerofugal neurons and thus may take part in activating sympathetic reflexes to the gut. (246 words).

POS-WED-026

ENHANCED GLUTAMATERGIC INPUTS TO PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS CONTRIBUTE TO INCREASED BLOOD PRESSURE AND SYMPATHETIC NERVE ACTIVITY IN CHRONIC KIDNEY DISEASE

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Increased sympathetic nerve activity (SNA) is a feature of chronic kidney disease, contributing to the associated hypertension and increased risk of mortality. The central cause of elevated SNA is unknown. Here we examined the role of the paraventricular nucleus of the hypothalamus (PVN) in the regulation of blood pressure (BP) and lumbar SNA in the Lewis polycystic kidney (LPK) rat, and control Lewis rats. The $GABA_A$ antagonist bicuculline (4mM) or non-specific glutamate receptor antagonist kynurenic acid (100mM) were bilaterally microinjected into the PVN, and BP and lumbar SNA recorded. Resting BP (systolic: 177.2 ± 7.5 vs. 117.5 ± 5.6 mmHg, P<0.001) and SNA (3.9 ± 0.4 vs. 2.26 ± 0.32 µV, P<0.01) were elevated in the LPK. Microinjection of kynurenic acid evoked a greater depressor response in the LPK (-27.6 ± 0.8 vs. -12.4 ± 0.6 mmHg, P<0.001). Microinjection of bicuculline evoked a markedly greater pressor (86.1 ± 5.8 vs. 47.7 ± 3.2 mmHg, P<0.01) and sympathoexcitatory response (144.4 ± 14.5 vs. 57.1 ± 17.2 % baseline, P<0.001) in the LPK, which was normalised by prior microinjection of kynurenic acid (51.4 ± 8.5 vs. 86.1 ± 5.8 mmHg, P<0.05; 65.9 ± 9.5 vs. 144.4 ± 14.5 % change baseline, P<0.001). These results indicate that in the LPK there is both glutamate-dependent and glutamate-independent GABAergic control of the PVN, and that glutamate-independent GABAergic control is unaltered. Glutamatergic control of the PVN, conversely, is enhanced in the LPK and may contribute to the hypertension observed in chronic kidney disease. (247 words)

POS-WED-027

THE INTERACTION BETWEEN ALCOHOL INDUCED NEUROINFLAMMATION AND DEPRESSIVE-LIKE BEHAVIOUR.

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There is well-established comorbidity between alcohol use disorders and depression. In many cases depression is a consequence of chronic stress and a resultant dysregulation of the HPA axis. Inflammatory cytokines are capable of activating the HPA axis. Interestingly, recent studies have identified a critical role of alcohol-induced neuroinflammation in the acute and chronic actions of alcohol. However, it is yet to be determined if alcohol-induced neuroinflammation influences the HPA axis and drives depressive behaviours. In this study male Balb/c mice were administered alcohol (2.5g/kg 20% v/v, i.p.) for 1, 3 or 5 day/s and were then subjected to either the forced swim test or allowed to remain in their home cage 24 h post final injection. Serum corticosterone concentrations were then measured at 30 mins post-FST. A subset of mice were also sacrificed at 1 or 3 h following their third dose of alcohol to determine its immediate effects. Mice exposed to 3 and 5 days of alcohol had significantly increased immobility time in the FST ($p<0.05$ compared to vehicle), which was related to increased serum corticosterone concentrations which peaked at 5 days of alcohol exposure ($p<0.0001$ compared to vehicle). This priming of the HPA axis to respond in an exaggerated manner to stress may relate to alcohol induced increases of IL-6 within the hypothalamus and hippocampus, peaking at 1 and 3 h post-alcohol injection respectively. Thus alcohol increases the response to acute stressors due to hyperactivity of the HPA axis, with further investigation needed to determine the exact mechanisms. (250 words).

POS-WED-028

INVOLVEMENT OF OREXIN IN METHAMPHETAMINE-INDUCED HYPOPHAGIA AND HYPERACTIVITY

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Methamphetamine is a psycho-stimulant drug and known to affect arousal level, behavioural activity and appetite. Orexin is a neuropeptide localized in hypothalamus and is know to play an important role in feeding behaviour and maintaining wakefulness. We investigated whether orexin was involved in methamphetamine-induced physiological response such as hyperactivity in orexin knockout (KO) mice and their wild type (WT). We provided methamphetamine containing drinking water (0.005%) for 21 days and assessed locomotor activity and food intake before/after the treatment and between KO and WT mice. Methamphetamine treatment increased locomotor activity by 140±30 % ($n=6$, $P<0.001$) in WT, but not in KO mice. Methamphetamine decreased food intake by 25±4% ($n=6$, $P<0.01$) during the first three days of treatment in WT mice, but not in KO mice. These data suggest that orexin contributes to methamphetamine-induced hypophagia and hyperactivity. (136 words).

POS-WED-029

Disruption of retinal amyloid beta homeostasis in glaucoma and the evidence of TrkB receptor mediated protection

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Levels of amyloid beta (Aβ) in the brain are regulated by its rate of production from amyloid precursor protein (APP) and by its rate of clearance. These levels are altered in several neuro-degenerative states such as Alzheimer's disease. In this study we sought to investigate alterations in the levels of Aβ (1-42) peptide in the retinas of rats subjected to experimental glaucoma. Tropomyosin related kinase B (TrkB) receptor activation is known to protect the retinal ganglion cells (RGCs) in glaucoma. The protective effects of TrkB receptor agonist 7,8 dihydroxyflavone in maintaining the Aβ homeostasis in glaucoma were investigated. A unilateral chronic ocular hypertensive model was established by weekly microbead injections in the adult SD rats. One group of rats was administered 7,8 DHF intraperitoneally for 20 weeks. Levels of Aβ were evaluated using specific ELISA kits. Exposure to increased intra-ocular pressure (IOP) resulted in an abrupt increase in the Aβ 1-42 species in the optic nerve head region of retina ($p<0.0001$). The group of rats that were exposed to increased IOP and administered TrkB agonist 7,8DHF depicted a significant decline of Aβ 1-42 levels ($p<0.001$). We have demonstrated that Aβ homeostasis is altered in the retina of animals exposed to experimental glaucoma. Pathological increase in Aβ can be reduced by administering an agonist of TrkB receptor. These findings should provide unique insights into preventative and/or therapeutic approaches to control neurotoxic Aβ levels in the retina in glaucoma and also in the brain in other neurodegenerative conditions. (246 words).

POS-WED-030

NEUROPROTECTIVE EFFECT OF POSTCONDITIONING ON EMBOLIC MODEL OF CEREBRAL ISCHEMIA IN RAT

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Objective: It has been reported that ischemic postconditioning, conducted by a series of brief occlusion and release of the bilateral common carotid arteries, confers neuroprotection in permanent or transient models of stroke. However, effects of postconditioning on embolic stroke have not been yet investigated.

Materials and Methods: In the present study, rats were subjected to embolic stroke (N=30) or sham stroke (N=5). Stroke animals were divided into control (N=10) or three different patterns of postconditioning treatments (N=20). In pattern 1 of postconditioning (PC10, N=10), the common carotid arteries (CCA) were occluded and reopened 10 and 30 sec, respectively for 5 cycles. Both occluding and releasing times in pattern 2 (PC30, N=5) and 3 (PC60, N=5) of postcoditionings, were five cycles of 30 or 60 sec, respectively. Postconditioning was induced at 30 min after stroke. Cerebral blood flow (CBF) was measured from 5 min before to 60 min after stroke induction. Infarct size, brain edema and neurological deficits and reactive oxygen species (ROS) level were measured 2 days later.

Results: While PC10 ($p<0.001$), PC30 and PC60 ($p<0.05$) significantly decreased infarct volume, only PC10 decreased brain edema and neurological deficits ($p<0.05$). Also PC10 prevented the hyperemia of brain at 35, 40, 50 and 60 min after the embolic stroke ($p<0.005$). No significant difference in ROS level was observed between PC10 and control group.

Conclusion: Based on our data, ischemic postconditioning reduces infarct volume and brain edema, decreases hyperemia after injury insult and improves neurological functions after the embolic model of stroke.
Key words: postconditioning, embolic stroke, cerebral blood flow

POS-WED-031

EVALUATION OF CIRCULATING ENDOTHELIAL MICROPARTICLES IN PATIENTS WITH ARTERIOVENOUS MALFORMATION (AVM)

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The development of new treatments for brain arteriovenous malformations (AVMs) requires a better understanding of their endothelial molecular biology, but this is difficult to study in human patients. Endothelial microparticles (EMPs) are a heterogeneous population that are released during activation or apoptosis. These are known to be markers of vascular injury and endothelial cell disturbance and could potentially be used as indicators of AVM endothelial molecular characteristics. In this study, EMP levels were evaluated in AVM patients and healthy volunteers.

Blood samples were taken from 9 AVM patients and 14 healthy volunteers. Stroke, cerebral malaria, multiple sclerosis, hypertension, brain tumour, idiopathic thrombocytopenic purpura and brain haemorrhage were exclusion criteria. Integrin V-alpha (CD51) and endoglin (CD105) were used as markers of endothelial injury for the flow cytometric analysis of the samples.

Increased levels of CD51 and decreased levels of CD105 were observed in the EMP antigenic profiles of AVM patients. These findings support the hypothesis that EMPs reflect endothelial changes in human AVMs and could be used to study their molecular biology. (222 words)

POS-WED-032

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DIFFERENTIAL PATTERNS OF CYTOKINE EXPRESSION IN THE NEURAXIS OF RATS WITH 'PAIN AND DISABILITY' FOLLOWING NERVE INJURY

Paul J. Austin, Annika M. Berglund, and Kevin A. Keay

School of Medical Sciences, University of Sydney, NSW, 2006.

Objective: Sciatic nerve chronic constriction injury (CCI) evokes '*pain*' (allodynia) in all rats, yet triggers changes in social behaviour in only a subpopulation, termed '*Pain and Disability*'. Studies have shown immune changes occur in the peripheral and central nervous systems following CCI. To determine whether the immune response following CCI was responsible for behavioural disability, we quantified the expression of multiple cytokines throughout the neuraxis. **Methods:** Rats underwent CCI or sham injury, and on the basis of changes in post-CCI dominance in social interactions were characterised as *Pain and Disability* (n=5) or *Pain alone* (n=5). Following sacrifice the following tissues were dissected; sciatic nerve, L4/L5 DRGs, L4/L5 spinal cord, hypothalamus, and contralateral hippocampus. Using a multiplex cytokine assay, expression of IL-1β, TNF, IL-6, IL-10, IL-17A, IFNγ and MCP-1 were quantified. **Results:** In the sciatic nerve of CCI rats there was a significant increase in IL-17A and MCP-1 compared to sham, whilst IL-6 was increased selectively in *Pain and Disability* rats. IL-6 and MCP-1 were significantly increased in the DRG and spinal cord of all CCI-rats compared to sham, whilst Il-1β was increased in only spinal cord. In the hippocampus, TNF and IFNγ were significantly increased in all CCI-rats compared to sham. In *Pain and Disability* rats, IL-10 and IL-17A expression were increased in the hippocampus and hypothalamus, respectively. **Conclusion:** These findings suggest that although an immune response occurs in all injured rats, *Pain and Disability* rats exhibit an anatomically specific cytokine expression pattern, which may be responsible for behavioural disability. (Word count: 250)

POS-WED-033

BEHAVIOURAL DISABILITY CORRELATES WITH IMMUNE CELL EXPRESSION BUT NOT MYELIN DISRUPTION FOLLOWING SCIATIC NERVE INJURY IN RATS

Paul J. Austin, Priya Kunjan and Sam Billyard, and Kevin A. Keay
School of Medical Sciences, University of Sydney, NSW, 2006.

Objective: Sciatic nerve chronic constriction injury (CCI) evokes sensory '*pain*' (allodynia) in all rats, but behavioural disabilities in only a subpopulation, termed '*Pain and Disability*'. Studies have shown immune cells invade the injured nerve and dorsal root ganglia (DRG) following injury, contributing to pain. To determine whether differences in axonal damage and/or the immune response following CCI were responsible for behavioural disability, we quantified disruption to myelination and the number of immune cells. **Methods:** Rats underwent CCI or sham injury, and due to injury-evoked changes in dominance behaviour were characterised as *Pain and Disability* (n=15) or *Pain alone* (n=16). S100, T cell receptor and ED1 expression in sciatic nerves and DRGs were analysed using immunohistochemistry. Nerves were also analysed for myelin thickness (1mm from injury site), using electron microscopy. **Results:** Myelin thickness decreased by ~20% after CCI, but did not correlate with CCI-induced disability, nor did expression of S100, a Schwann cell myelination marker. CCI increased T cell numbers in sciatic nerves ($P<0.05$) and DRGs ($P<0.05$) compared to sham. Furthermore, T cell number positively correlated with behavioural disability ($R^2=0.32$). CCI increased ED1 macrophage number in the nerve ($P<0.001$), being greatest in *Pain and disability* rats, although this was not significantly greater than *Pain alone*. **Conclusion:** Following nerve injury more T cells and macrophages are found in the nerves of *Pain and Disability* rats, although they have similar axonal damage as *Pain alone* rats. Therefore, *Pain and Disability* rats have a more pronounced immune response, which may contribute to behavioural disability. (Word count: 250)

POS-WED-034

A POSSIBLE NOVEL IMMUNE ROLE FOR NOGO-RECEPTOR 1 IN A B-CELL POPULATION LOCALISED IN THE CNS DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is a neuroinflammatory disease of the spinal cord, brain and optic nerve manifesting as demyelination and progressive neurodegeneration. Data from our laboratory have demonstrated that deletion of the cognate receptor for Nogo-A, Nogo receptor 1 (NgR1), can protect against axonal degeneration and thus progression of disease, in mice induced with MOG_{35-55} to manifest experimental autoimmune encephalomyelitis (EAE). However, what is currently unknown is whether there exists a prominent immunological role for NgR1. We performed flow cytometric analysis on dendritic cells, T - and B-lymphocytes that were isolated from the spleens, lymph nodes and spinal cords from both *ngr1* $^{-/-}$ and *ngr1* $^{+/+}$ littermate mice (n=52 mice) at different clinically defined stages of EAE disease. We observed an augmented response in the immature B cell population from *ngr1*$^{+/+}$ littermate mice with the onset and progression of the disease. These cells were specifically localised within small follicles in submeningeal regions of the spinal cord at the onset and peak stages of EAE. This population of cells could not be demonstrated within the spinal cords of EAE-induced *ngr1* $^{-/-}$ mice or during the chronic stage of disease in wild-type littermate mice. Our results emphasise that the infiltration of B-cells into CNS is NgR1-dependent and may implicate this population of cells during the progression of neuroinflammatory-mediated degeneration, commonly observed during the progressive phase of MS. (220 words).

POS-WED-035

SUPPRESSOR OF CYTOKINE SIGNALLING 2 (SOCS2) OVEREXPRESSING MICE HAVE AN INCREASED INFLAMMATORY CELL RESPONSE AFTER TRAUMATIC BRAIN INJURY

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Suppressor of cytokine signalling 2 (SOCS2), a negative regulator of cytokine signalling, was identified in our lab to enhance neurite outgrowth in neural cell culture and increase the survival of newborn adult hippocampal neurons under basal physiological conditions in-vivo. We are now studying the effects of SOCS2 overexpression on neurogenesis, newborn neuron survival and cellular composition of the brain following traumatic brain injury (TBI).

SOCS2Tg mice on a NestinCreER^{T2}/eYFP background and littermate wildtype controls were subjected to mild controlled cortical impact or sham surgery. This background allows neural precursor cell (NPC) specific eYFP expression upon tamoxifen exposure. Tamoxifen was administered 7d prior to injury to label pre-existing NPCs. Additionally, EdU was administered immediately after injury to label NPCs and other proliferative cells. Tissue was collected 35d post-TBI and assessed via immunohistochemistry. Equal numbers of newborn neurons arising from the eYFP^{+} population were found in the cortex of sham and injured animals of both genotypes. However, no EdU^{+}/NeuN^{+} neurons were identified in sham or injured animals of either genotype. Despite no detectable changes in newborn neuron survival post-injury, there was a two fold increase in the total number of EdU^{+} cells present in the injured cortex of SOCS2Tg animals compared to wildtype. CD11b^{+} microglia/macrophages were the major contributors to this increase. EdU^{+}/Olig2^{+} were also present and their number was significantly, but not differentially, increased post-TBI in both genotypes. Thus far, our results suggest a role for SOCS2 in modulating the inflammatory cell population after TBI. (244 words).

POS-WED-037

FORMATION OF A TRUNCATED FRAGMENT OF SRC (SRCΔN) FOLLOWING ISCHEMIC STROKE IN RATS

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Excitotoxicity resulting from overstimulation of glutamate receptors (GluR) is a major cause of neuronal death in ischemic stroke. Overstimulated GluR exerts neurotoxicity through overactivation of calpains by catalysing limited proteolysis cellular proteins such as Src, a neurotrophic enzyme under normal physiological condition. Calpain cleavage of Src leads to expression of SrcΔN ultimately causing neuronal death. In this study we sought to determine the timepoints in which SrcΔN presents itself following ischemic stroke. ET-1 stroke-induced rats were sacrificed 1,3,6 and 24hrs following stroke along with sham-treated rats (n=4/group). Brains were harvested, separated into its hemispheres and further sectioned into three for precise analysis. Our findings show that full-length Src was present in all sham-treated and stroke-induced brain lysates with a significantly decreased expression levels (~10% compared to contralateral side) detected in lysates from the 1hr group but not in 3,6 or 24hr group. Presence of SrcΔN (~52kDa) was detected only in ipsilateral side of stroke-induced brain lysates in a time-dependent manner. Expression levels of SrcΔN were markedly increased in the 24hrs group compared to earlier timepoints. Findings here suggest a new function of Src where calpain cleavage acts as a molecular switch converting Src from a cell survival promoter to neuronal death mediator. Our discovery of the neurotoxic action of the truncated Src fragment suggests new therapeutic strategies with potential to minimize brain damage in ischemic stroke. (226 words)

POS-WED-038

THE ROLE OF NEUROGENIN 2 IN APP-STIMULATED NEURAL STEM OR PROGENITOR CELLS DIFFERENTATION.

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The amyloid precursor protein (APP) is well studied for its role in Alzheimer's disease. However, little is known about its normal function. APP may be important for neuronal growth and differentiation. APP expression increases as neural stem or progenitor cells (NSPCs) mature into neurons. Our recent work shows that APP stimulates NSPCs proliferation and neuronal differentiation. The effect on proliferation is mediated by cystatin C which is secreted and can act as an autocrine growth factor. However, our results show that the differentiation of NSPCs into neurons cannot be completely explained by this factor. There are likely to be other factors involved in the regulation by APP-induced differentiation of NSPCs. In order to understand how APP controls differentiation of NSPCs, we used a gene array approach to identify differences in the genes involved in neurogenesis between NSPCs from APP-KO mice and from WT background strain controls. We found that neurogenin2 (Ngn2), a basic helix-loop-helix transcription factor, is highly downregulated in NSPCs from APP-KO mice. Ngn2 plays an important role in neurogenesis, increasing the expression of proneural genes and driving neural fate by inhibiting expression of glial genes in NSPCs. Indeed, the expression of Ngn2 in APP-KO NSPCs transfected with a pCAG-Ngn2-IRES-GFP construct produced an increase in neuronal morphology compared to control cells. Taken together, our results support the idea that the APP-stimulated NSPCs differentiation could be due, at least in part, to the expression of Ngn2.

POS-WED-039

EXPRESSION OF THE NEURITE OUTGROWTH INHIBITOR NOGOA FOLLOWING FOCAL ISCHEMIA OF PRIMATE NEOCORTEX SWITCHES FROM ASTROCYTES IN THE NEONATE TO NEURONES IN THE ADULT

Boghdadi A, Bourne JA

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Purpose: The primary visual cortex (V1) is commonly affected by ischemic stroke with molecular and cellular responses differing between the developing and adult brain. Inhibitory molecules such as NogoA may have key roles in repair inhibition following neocortical injury. We investigated the cellular expression of NogoA at the lesion site following ischemic injury of neonate and adult marmoset V1.
Methods: To induce ischemia, injections of 0.5µL (1mg/mL) Endothelin-1 were performed over 4 sites surrounding the posterior cerebral artery of operculum V1 in neonate (PD14; n=3) and adult (>1 year; n=3) marmosets. Following 3 weeks recovery immunofluorescent labelling with primary cell type markers was performed on control (PD14; n=2, adult; n=2) and lesioned hemispheres to define cell subtypes expressing NogoA.
Results: Immunohistochemistry revealed NogoA on oligodendrocytes (RIP), neurones (NeuN) and astrocytes (GFAP) in both neonate and adult marmosets post injury. NogoA expression was negligible in neonatal control neocortex but was found to be significantly expressed by astrocytes post-injury in the lesion core. Interestingly, in the adult lesion core, NogoA was identified at high levels on a small population of excitatory neurones (EAAT3).
Conclusion: This shift in expression from astrocytes to neurones may implicate a differential role for NogoA on these cell types between a developing and a mature brain following injury. We postulate that NogoA on astrocytes may contribute to astroglial inhibition of neurite outgrowth and that neuronal NogoA may play modulatory roles following ischemic injury, such as redirecting putative regenerating neurites away from the metabolically compromised injury site. (248 words)

POS-WED-040

NEUROTRANSMITTER DYSFUNCTION IN SUDDEN INFANT DEATH SYNDROME (SIDS)

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Purpose: SIDS is one of the most significant causes of post neonatal mortality in developed countries. Abnormal neurotransmitter levels in brainstem respiratory centres have been identified in the pathogenesis of SIDS, specifically involving serotonin (5HT). As the respiratory network is defined by an extensive array of multiple neurotransmitters systems, it is considered unlikely that 5HT alone is involved in the pathogenesis of SIDS. This study aimed to further the understanding of the role of neurotransmitters in SIDS by evaluating the expression of substance P (SP), another key excitatory respiratory neurotransmitter and its receptor (NK1), in SIDS infants compared to non-SIDS controls. **Methods:** Brainstem sections from SIDS and control cases from x cases underwent immunohistochemical evaluation for substance P and Neurokinin 1 (NK1). **Results:** Compared with non-SIDS controls, there was a significant decrease in substance P immunoreactivity in SIDS cases ($p<0.05$) in addition to a reduction in staining for the main receptor for SP, NK1 receptor levels ($p<0.01$) in a key respiratory region of the brainstem, the arcuate nucleus. **Conclusion:** Abnormal levels of neurotransmitters within critical respiratory centres in the infant brainstem have become increasingly recognised as a major factor in the pathophysiology of SIDS. This study has demonstrated significantly reduced levels of SP and NK1 in the arcuate nucleus of SIDS infants. Reductions in SP and NK1 immunoreactivity may be considered a sign of chronic hypoxia. Such findings suggest that brainstem failure in SIDS infants may involve additional neurotransmitters to 5HT, a concept that requires further investigation.

POS-WED-041

THE NEURONAL RESPONSE TO INJURY. IS IT SUBTYPE SPECIFIC?

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Relatively few studies have addressed the role of interneurons in the degenerative and regenerative responses evoked by traumatic brain injury (TBI). Previous studies in our group indicate that excitatory and inhibitory cortical neurons respond differently to trauma. To further investigate the response to trauma evoked by these two populations we utilised an *in vitro* model of direct transection/scratch injury. Primary cultures of neocortical neurons derived from E15.5 thy-1 YFP positive pups were grown to relative maturity (15 days *in vitro*), injured and the response to injury of CR immunolabelled interneurons and YFP positive pyramidal cells was evaluated at 0, 4 and 24 h post injury (PI) at increasing distances from the injury site. A minimum of 25 cells per time point and per distance from the injury derived from 3 separate neuronal cultures were traced with Neurolucida and a segment dendrite x-y angle analysis, categorising neurites as either directed toward the injury site or away from the injury site, was performed. Results demonstrated a significant ($p<0.05$ unpaired Ttest) increase in the mean number of neurites from CR positive interneurons rearranged away from the injury at 4h PI proximal to the injury site whereas YFP positive pyramidal neurons did not undergo any significant remodeling in response to the injury within the same time frame. Our results highlight the importance of having a better understanding of the response to injury yielded by specific cellular subtypes. (250 words).

POS-WED-042

IFNAR-1 SIGNALLING REGULATES THE MICROGLIAL PHENOTYPE IN RESPONSE TO PERIPHERAL LPS CHALLENGE

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Neuroinflammation has been proposed to contribute to the neuronal cell damage following injury, infection or disease. We hypothesise that type-1 interferon (IFN) signalling is a key mediator of the neuroinflammatory response. Mice lacking the type-1 IFN receptor (IFNAR1) (n=6) were given a single intraperitoneal (I.P) injection of lipopolysaccharide (LPS) (2mg/kg). A temporal analysis of the early neuroinflammatory changes was undertaken by QPCR, western analysis and immunohistochemistry. QPCR confirmed increased expression of IFN-β and the downstream mediator, IRF7 in wildtype mice at 6 and 24 hours post-LPS injection (compared to sham levels), a response that was decreased in the IFNAR1-/- brains. Increased IBA-1 immunoreactive microglial cells were identified in the substantia nigra of both wildtype and IFNAR-1-/- mice at 6 and 24 hours post-LPS injection. Significantly, at both time-points the microglial number and activation was reduced in the IFNAR1-/- brains compared to wildtype mice. No difference in the mRNA levels of the pro-inflammatory cytokines IL-6, TNF-α and IL1-β□between wildtype and IFNAR1-/- brains. Upon activation, microglia can polarise to form two differing phenotypes; M1 (damaging) or M2 (neuroprotective). QPCR identified reduced expression of both M1 (iNOS and CD32) and M2 (Arg-1, YM1, CD206 and TGF-β□ microglial markers in the IFNAR1-/- brains at 6 hours post-LPS suggesting an altered neuroinflamatory phenotype. This study confirms type-1 IFN signalling plays a role in regulating the cellular environment involved in the neuroinflammatory response. This modulation has implications in both acute and chronic neuropathologies where neuroinflammation may be a critical event in perpetuating the neuronal cell death. (250 words)

POS-WED-043

PLASMA BIOMARKERS IN PRESENILIN1 MUTATION CARRIERS USING ISOBARIC TAG FOR RELATIVE AND ABSOLUTE QUANTITATION (iTRAQ).

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Objective: Currently a post-mortem autopsy is the only definitive diagnosis for Alzheimer's disease (AD), while pre-mortem biomarkers in the cerebrospinal fluid and neuroimaging are invasive and uneconomical. This study compares plasma protein concentrations in individuals destined to AD (carry a mutation responsible for autosomal dominant AD; MC) with those who are non-carriers of the mutations (NC), using isobaric tag for relative and absolute quantitation (iTRAQ). The study findings contribute towards identifying candidate proteins for the development of an early diagnostic blood test biomarker panel, by measuring pathogenic changes occurring from the early stages of this progressively neurodegenerative disease.

Methods: Participants belonged to Perth and Melbourne sites of the multicentre Dominantly Inherited Alzheimer's Network (DIAN) study. Based on clinical dementia rating (CDR) score, plasma from seven pre-symptomatic Presenilin1 MC (CDR=0), seven symptomatic Presenilin1 MC (CDR>0) and seven NC were pooled into three independent groups, respectively. The three pools underwent immuno-depletion and were analysed using the 4-plex iTRAQ approach.

Results: 327 proteins from 11081 distinct peptides were identified and relatively quantified. We observed 15 down-regulated and 3 up-regulated plasma proteins in both pre-symptomatic and symptomatic Presenilin1 MC compared to NC. These differentially expressed proteins are associated with inflammatory, immunological, blood coagulation and lipid transport biological pathways.

Conclusion: The differentially expressed proteins identified will have to be verified using the multiple reaction monitoring method. Verification of these candidate proteins will give insight into the complex inter-related biological changes occurring before and during expression of AD phenotype.(243 words).

POS-WED-044

EFFECTS OF DIAZEPAM ON THE HIPPOCAMPAL EEG AND HISTOLOGY IN THE KAINIC ACID MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

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Mesial temporal lobe epilepsy (MTLE) is the epilepsy most commonly resistant to anti-epileptic drugs. A characteristic feature of this disease is its long seizure free period during which epileptogenic processes occur. Our study aimed to establish the intra-hippocampal kainic acid model of MTLE in C57Bl6 mice to study epileptogenesis using chronically implanted electrodes and EEG recordings. We subsequently used this model to investigate the effect of diazepam administration during the epileptogenic period on the MTLE phenotype. Kainic acid injected (50 nl, 20 mM, AP -2.0, ML -2.0, DV -2.0) mice displayed a chronic phenotype of frequent episodes of spike trains and seizure bursts in the hippocampus ipsilateral to the injection site. Histological examination revealed kainic acid injection caused marked CA cell loss and dentate gyrus dispersion in the ipsilateral hippocampus. Diazepam administration appeared to improve this chronic phenotype with the total number of individual spikes reduced from 4780/hr to 715/hr ($p < 0.05$, n=4-5 mice). Although we are still optimizing the objective variables used to define bursts of events as seizures, the incidence of mice with such seizures was reduced from 4/4 in kainic acid treated mice to 1/5 in kainic acid and diazepam treated mice. Typically, the hippocampal damage was markedly reduced in most diazepam treated mice. This is consistent with a loss of inhibitory signalling during the early epileptogenic period contributing to the chronic MTLE phenotype. (228 words)

POS-WED-045

EXCITATORY NEURONS IN THE ADULT MOUSE NEOCORTEX DEMONSTATE RESILIENCE TO MILD STRUCTURAL INJURY AND CAPACITY FOR ADAPTIVE REMODELLING

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Injury to the adult brain may result in long-term impairment in cognitive and motor function. Ongoing adaptive remodelling of neuronal circuitry is postulated to contribute towards recovery of function, however the cellular mechanisms underlying this process are not fully understood. Using a clinically relevant mouse model of mild traumatic brain injury, fluid percussion injury (FPI), we are investigating the cellular mechanisms linking structural alterations with functional recovery. Adult male YFPH mice, expressing yellow fluorescent protein in layer 5 cortical pyramidal neurons underwent mild lateral FPI (1.61±0.1atm). Brain injury caused apnoea (12.50±2.88secs) and significantly longer righting time (3.91±1.98mins) relative to sham-operated animals (0.14±0.12secs). In one cohort of animals a cranial window was constructed over the injury site for in vivo two-photon imaging. A second cohort of animals was perfused one week following FPI/sham-operation and processed for histological analysis. Preliminary in vivo imaging studies demonstrated long-term survival (> 4 months) of YFP neurons following FPI. Surviving neurons underwent apical dendrite spine addition and elimination. Analysis of fixed tissue showed there was significantly more ($p<0.05$) axon pathology (size and number of YFP degenerating axonal beads) in the ipsilateral corpus callosum of brain-injured relative to sham-operated animals, however there was no significant reduction in cell number and relatively normal cytoarchitecture was maintained. Together our data demonstrate that projection neurons in the adult mouse cortex exhibit resilience to mild structural injury and an ongoing capacity for adaptive remodelling. Our future studies aim to elucidate the therapeutic potential of microtubule stabilising agents in this response.

249 words

POS-WED-046

PLAQUE DYNAMICS FOLLOWING FOCAL BRAIN INJURY IN APP/PS1 MICE

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Objective: Epidemiological studies have demonstrated brain injury as a risk factor for the development of Alzheimer's disease. The purpose of this study was to examine plaque dynamics following focal cortical brain injury in 9-month-old APP/PS1 mice.
Methods: Nine-month old APP/PS1 and C57/Bl6 male mice underwent focal cortical brain injury and were sacrificed at 24 hours and 7 days post injury (PI) (n=3 per group). A burr hole was drilled into the skull (3mm lateral, 1mm posterior to bregma), and a 26-gauge needle lowered to a depth of 1mm into the cortex for 10 minutes. Amyloid beta plaques were immunolabelled with the antibody MOAB-2 in 50µm tangential sections. Furthermore, microglial, axons and synapses were immunolabelled with Iba-1, SMI-312 and synaptophysin respectively.
Results: No plaques were seen in wild-type C57/Bl6 mice at any timepoint. There was a significant increase in MOAB-2 labelled amyloid beta plaque load surrounding the injury site at 24 hours PI in injured mice compared to sham injured controls (1.21% vs 0.55%, p-value 0.03). However, at 7 days PI there was no difference in MOAB-2 plaque load in injured versus sham injured control mice (0.9% vs 0.78%, p-value 0.76).
Conclusion: In 9-month old APP/PS1 mice with established amyloidosis, a focal cortical brain injury increases local plaque load acutely, however this increased plaque load is not sustained at longer PI timepoints (Word count 250).

POS-WED-047

SUBSTANCE P AND TAU PHOSPHORYLATION FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY

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Repeated mild traumatic brain injury (mTBI), is associated with the development of the neurodegenerative disorder, chronic traumatic encephalopathy (CTE). The characteristic histopathological feature of CTE is the accumulation of hyperphosphorylated tau. It is thought that the neuropeptide substance P (SP) may play a role in the development of this tau phosphorylation, as it is released following mechanical injury and activates kinases implicated in tau phosphorylation. Furthermore, repeated activation may promote greater release of SP. As such, levels of SP and hyperphosphorylated tau within the cortex were examined 24 hrs following graduated diffuse impact acceleration injury, with a 450g weight released from 0.5, 1 and 2m onto a metal helmet. Three repeated injuries spaced 5 days apart were performed at the 0.5 and 1m heights. The results indicated a threshold of mechanical stimulation was required to see a significant increase in SP expression following a single impact, with only the animals subjected to the injury from 2m demonstrating significantly elevated SP. However with repetitive injury, a 2-fold and 3-fold increase in the number of SP positive cells respectively, were seen following impact from 0.5 and 1m compared to a single impact from that height. Examination of neurons positive for hyperphosphorylated tau revealed similar levels in animals receiving a repeated 1m injury compared to those receiving only one more severe 2m injury, with all other groups comparable to shams. This demonstrates that SP release can be primed following repetitive mTBI, and its release is associated with increased levels of phosphorylated tau. (249 words)

POS-WED-048

BLOOD-BRAIN BARRIER BREAKDOWN IN TRAUMA: SUBSTANCE P AND THE TRANSCELLULAR VERSUS PARACELLULAR PATHWAYS.

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Recent research has linked the neuropeptide Substance P (SP) with increased blood-brain barrier (BBB) permeability in a variety of neurological disorders, including traumatic brain injury (TBI). The SP receptor NK1 is present on both endothelial cells and caveolae, suggesting a connection with both the paracellular and transcellular pathways of BBB transit. However, the relative contribution of these pathways to BBB dysfunction in specific varieties of TBI is largely unknown. The present study seeks to elucidate the activity in each pathway following TBI, and investigate their relationship to SP expression and BBB integrity. Using archival ovine tissue retained from animals sacrificed at 5 hours following non-missile impact-acceleration TBI or non-accidental head injury (NAHI), the expression of albumin, SP, caveolin-1, and claudin-5 were evaluated immunohistochemically alongside standard neuropathological stains. In all injured animals, increased BBB permeability was demonstrated via increased albumin immunostaining. Furthermore, regardless of injury type, increased perivascular SP and caveolin-1 was observed while claudin-5 was preserved at uninjured levels. Thus, in the early phase post-TBI, SP may participate in facilitating albumin flux through the transcellular pathway via activation of caveolae, rather than opening tight junctions to allow paracellular permeation. Future studies utilising NK1 receptor antagonists will further enhance our understanding of the role of SP in this process. (212 words)

POS-WED-049

ACUTE OLANZAPINE AND HALOPERIDOL TREATMENT PROMOTES BDNF SIGNALLING: POTENTIAL MECHANISMS FOR IMPROVING COGNITIVE FUNCTION IN SCHIZOPHRENIA

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Evidence suggests that abnormal activity of brain-derived neurotrophic factor (BDNF) may contribute to alterations in hippocampal function and hippocampal-dependent learning and memory in patients with schizophrenia. **Objective:** This study investigated the effects of olanzapine and haloperidol on BDNF/TrkB/PI3K/Akt/GSK3ß signalling pathways in the hippocampus for understanding their possible pharmacological effects on cognition in schizophrenia. **Methods:** Female rats were treated with haloperidol (0.1mg/kg), olanzapine (1.0mg/kg) or saline for one times, or three times/day for 3 or 6 days. Western blot was employed to determine total and phosphorylated protein levels of BDNF, TrkB, pTrkB, Akt, pAkt, pGSK3ß and GSK3ß in the hippocampus. **Results:** Haloperidol treatment significantly increased phosphorylation of TrkB and GSK3ß^{Ser9}, while olanzapine significantly increased phosphorylation of GSK3ß^{Ser9} after a single injection. At the three days time-point, haloperidol significantly increased phosphorylation of AKT^{Ser473} and GSK3ß^{Ser9}. Olanzapine significantly increased phosphorylation of GSK3ß^{Ser9} and TrkB. Furthermore, both antipsychotics significantly increased phosphorylation of AKT^{Ser473}, TrkB and GSK3ß^{Ser9} at the six days time-point. Also, the total AKT protein levels were significantly increased by haloperidol and had a tread to be increased by olanzapine at this time-point. **Conclusion:** This study supported that acute olanzapine and haloperidol administration might have potential effects for improving cognitive function in schizophrenia. (200 words).

POS-WED-050

GLIAL CELL ACTIVATION IN PERI-INFARCT TISSUE IN A PHOTOTHROMBOTIC MODEL OF STROKE IN RATS

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Early responses of microglia and interactions of these cells with astrocytes in peri-infarct tissue following stroke lead to the development of changes, including astrocytic secretion of chondroitin-sulphate proteoglycans, that can limit local neuronal plasticity and impede functional recovery. Although multiple aspects of the glial responses have been examined, few studies have focused on the early events that result in the generation of this inhibitory environment. There have also been few attempts to identify measures that can reliably quantify these cellular changes. In this study, approaches we recently developed based on immunohistochemistry of Iba1 (microglia /macrophages) and vimentin (astrocytes) were combined with Western blots to characterise responses of these cells during the first week following induction of photothrombotic stroke. Changes in microglial morphology associated with activation developed rapidly (by 3 hours) in peri-infarct tissue. These increased further and transiently spread to more distant cortical regions at 24 h. Changes in Iba1 immunoreactivity indicated initial losses of microglia from peri-infarct tissue followed by large increases up to 7 days. Analysis of vimentin expression suggested little activation of peri-infarct astrocytes at 24 h but dramatic increases at 2 days which progressed further at later times. Full-length neurocan, a key inhibitory proteoglycan, was also little expressed at 24 hours but increased markedly by 3 days. This study delineates key features of the distinct profiles of activation of peri-infarct microglia and astrocytes in response to photothrombotic stroke and identifies measures that can be used to evaluate treatments targeting these cells to promote recovery. (248 words)

POS-WED-051

SPHINGOSINE 1-PHOSPHATE LINKS APOE GENOTYPE AND ALZHEIMER'S DISEASE PATHOGENESIS

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The greatest genetic risk factor for late-onset Alzheimer's disease (AD) is the ε4 allele of Apolipoprotein E (ApoE). ApoE directly regulates secretion of the potent neuroprotective signaling lipid Sphingosine 1-Phosphate (S1P). S1P synthesis is catalysed by sphingosine kinases 1 and 2 (SphK1 and 2), and SphK1 positively regulates glutamate secretion and synaptic strength in hippocampal neurons. Using liquid chromatography-tandem mass spectrometry, we quantified S1P levels in six brain regions that are differentially affected by AD pathology, in a cohort of 34 post-mortem brains, divided into four groups based on Braak neurofibrillary tangle staging. S1P declined with increasing Braak stage, and this was most pronounced in brain regions most heavily affected by AD pathology. Mean S1P levels were 66% and 64% lower in Braak stage III-IV hippocampus (p=0.010) and inferior temporal cortex (p=0.014), respectively, compared to controls. Both SphK1 and SphK2 activity declined with increasing Braak pathology in the hippocampus (p=0.032 and 0.047, respectively), providing the enzymatic basis for loss of S1P. S1P levels were not correlated with amyloid beta peptide in human hippocampus, nor were they reduced in a mouse model of amyloidosis, indicating that amyloid production does not drive S1P loss. Hippocampal S1P levels were 2.5-fold higher in ApoE2 carriers compared to ApoE4 carriers, and multivariate regression showed a significant association between ApoE genotype and hippocampal S1P (p=0.0495), suggesting a new link between ApoE genotype and pre-disposition to AD. Recent approval of the drug Fingolimod, an S1P mimetic, for relapsing multiple sclerosis highlights the therapeutic relevance of this study. (250 Words)

POS-WED-052

LRRK2, A GENETIC LINK BETWEEN INFLAMMATION AND PARKINSON'S DISEASE.

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In recent years a number of genetic causes of Parkinson's disease (PD) have been discovered. Amongst these, mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) protein are a particularly common cause of autosomal-dominant inherited PD. Mutations in LRRK2 can increase the enzymes kinase activity. Consequently, a number of LRRK2 kinase inhibitors are in development as potential PD therapeutics. Stalling the translation of these inhibitors to trials is the current limited understanding of the physiological function of LRRK2. Our work has uncovered that LRRK2 is a novel component of the innate immune toll-like receptor (TLR) inflammatory pathway. Activation of the TLR pathway results in the production and secretion of a number of inflammatory cytokines, many of which are found increased in PD patients. To understand how LRRK2 effects inflammatory signalling we have studied LRRK2 deficient mice. These mice show surprising overlaps with aspects of immune dysfunction in Parkinson's disease patients. Moreover, by stimulating immune cells from LRRK2 deficient mice with agonists of TLR signalling, we have uncovered a novel role for LRRK2 as a negative regulator of interferon production. When coupled with our measurements of LRRK2 in blood and brain samples from PD patients these results may provide new insight into PD pathogenesis. (202 words)

POS-WED-053

CNS PLASTICITY IN PRECLINICAL MODELS OF INFLAMMATORY-MEDIATED GASTROINTESTINAL PAIN: A SYSTEMATIC REVIEW

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Abdominal pain frequently accompanies inflammatory disorders of the gastrointestinal tract (GIT). In order to understand the underlying CNS mechanisms, various animal models of visceral inflammation have been developed. We conducted a systematic review of preclinical evidence for CNS plasticity in animal models of visceral pain following GIT inflammation. We identified 208 articles, and selected 79 for detailed analysis. Rats were the most widely used species (76%). Most studies used adult animals (42%) with a strong bias towards males (74%). Behavioral (58%), anatomical/molecular (44%) and physiological (24%) approaches were used alone or in combination to assess CNS plasticity/involvement during or after GIT inflammation. The time points at which CNS changes were measured varied widely (< 1hr - > 2wks). Blinded outcomes were employed in 42% of studies, randomization in 10% and proof of visceral inflammation in 54%. Together the included studies indicate there is strong evidence for CNS plasticity following inflammation of the GIT, specifically in dorsal horn neurons. This evidence includes altered visceromotor responses and indices of referred pain, elevated neural activation and peptide neurotransmitter content, and increased excitability/altered discharge of dorsal horn neurons. Thus, current evidence supports continued use of these approaches in preclinical studies. There is substantial scope to improve aspects of study design such as randomization and blinded assessment, and to undertake systematic investigations to determine the influences of animal characteristics and experimental time-points, amongst others. Additionally, our knowledge of neuron populations involved in spinal cord plasticity and the precise physiological mechanisms involved is limited. (248 words)

POS-WED-054

THE N170 AND FACE PERCEPTION IN PSYCHIATRIC AND NEUROLOGICAL DISORDERS: A SYSTEMATIC REVIEW

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Face identity and facial expression recognition deficits have been documented in multiple psychiatric and neurological disorders. The N170 and VPP event-related potential (ERP) components and analogous M170 MEG component index neural processes associated with structural face processing, abnormalities of which may underlie social deficits. This review aimed to systematically assess the evidence for abnormalities in visual face processing as indexed by the N170/VPP/M170 in neurological and psychiatric disorders. 1707 papers were identified using PsychINFO and PubMed databases using the search terms (face OR facial OR faces) AND (ERP OR ERF OR “event related” OR “evoked potential”) AND (VPP OR “vertex positive” OR N170 OR N1 OR M170). Fifty-eight studies representing fourteen psychiatric/neurological disorders were selected for review, which employed an array of tasks to measure the N170 or VPP. None of the included studies measured the M170. There was some evidence for N170/VPP differences to upright faces in limited disorder groups (ADHD, Alcoholism, Alzheimer’s Disease and Mild Cognitive Impairment, Bipolar Disorder, Schizophrenia) and little evidence of differences in others (Autism Spectrum Disorders, Bulimia Nervosa, Major Depressive Disorder, Parkinson’s Disease, Prosopagnosia, Social Phobia). The divergence in between-group results across studies is likely to reflect high between-subject variance in N170/VPP measures. Within-subject experimental manipulations more consistently suggested abnormalities in face processing in disorders. The face inversion N170/VPP effect was predominantly absent in adults with Prosopagnosia and Schizophrenia and children with Autism Spectrum Disorders, suggesting that within-subjects task or stimuli manipulations are more sensitive to atypical face processing than direct between-group comparisons. (249 words).

POS-WED-055

THE INCIDENCE OF UNEXPECTED PATHOLOGY IN SA BRAIN BANK CASES: 10 YEAR CASE REVIEW

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This study reviews the findings from routine neuropathological examination of brains donated to the SA Brain Bank from 2001-2012 (n=186). Such examination is required for definitive diagnosis of many neurodegenerative illnesses but may also demonstrate pathologies that were not suspected during life. We examined the correlation between clinical and neuropathological diagnoses and the role of multiple pathologies. Data was assessed on the basis of expected pathology, additional pathology, unexpected pathology and clarification of uncertain pathology. Some cases satisfied all four criteria.

Findings were analysed in two 5-year periods, 2001-2006 and 2007-2012 to determine changes over time. Between 2001-2006, 81% of pathology confirmed the clinical diagnosis (n=84), increasing to 92% in the 2007-2012 period (n=102). Between 2001-2006, 48% of cases showed additional pathology rising to 67% in 2007-2012. The incidence of unexpected pathology (24-19%) and clarification of uncertain pathology (43-38%) remained relatively stable.

The clinical diagnosis of neurodegenerative diseases within this cohort appears to have become increasingly more accurate over the last 10 years, however the incidence of additional unexpected pathology has risen. These findings highlight the challenge of clinical diagnosis in these patients as well as the importance of formal neuropathological examination, which allows possible patterns of disease to be identified, potentially opening new avenues for research into aetiology and treatment. Whilst the incidence of brain diseases is increasing with the ageing population, autopsy rates are falling, however brain banks provide a pathway by which valuable information regarding the aetiology and pathogenesis of these devastating conditions may be obtained. (249 words)

POS-WED-056

ANALYSING THE ROLE OF HYPOXIA IN AUTOPHAGY USING ZEBRAFISH EMBRYOS.

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Autophagy is the principle pathway in a cell involved in clearing damaged proteins and organelles. Therefore autophagy is necessary to maintain turnover balance of peptides and homeostasis. Autophagy occurs at basal levels under normal conditions but can be upregulated by chemical inducers or stress conditions. Zebrafish (*Danio rerio*) serves as a versatile tool to understand the function of genes implicated in autophagy. We report the identification of the zebrafish orthologue of mammalian genes *MAP1LC3A (map1lc3a)* and *MAP1LC3B (map1lc3b)* by phylogenetic and conserved synteny analysis and examine their expression during embryonic development of zebrafish. Analysis of *map1lc3a & map1lc3b* function in zebrafish embryogenesis may be valuable for understanding their role in autophagy and the interplay of autophagy and other pathways in AD. We have also validated the LC3II/LC3I immunoblot assay in the presence of chloroquine (a proteolysis lysosomal inhibitor). We found that the LC3II/LC3I ratio is significantly increased in the presence of sodium azide treatment supporting that hypoxia induces autophagy in zebrafish. This was supported by our qPCR assay that showed an increase in *map1lc3a* transcript levels in the presence of sodium azide. Our study thus identifies the zebrafish orthologues of *MAP1LC3A & MAP1LC3B* and supports that hypoxia induces autophagy.

POS-WED-057

LAMP2 PROTEIN ISOFORMS ARE DIFFERENTIALLY AFFECTED IN EARLY PARKINSON'S DISEASE

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Lysosomes are the primary catabolic compartment for the degradation of intracellular proteins through autophagy. The presence of abnormal intracellular α-synuclein-positive aggregates in Parkinson's disease (PD) indicates that the degradative capacity of lysosomes is impaired in PD. Specific dysfunction of chaperone-mediated autophagy (CMA) in PD is suggested by reductions in the membrane receptor LAMP2A, although whether LAMP2A is the only LAMP2 isoform affected by PD is currently unknown. We used quantitative PCR and Western blotting to assess the mRNA and protein expression of all three LAMP2 isoforms in brain regions with and without PD-related increases in α-synuclein in autopsy samples from subjects in the early pathological stage of PD with increased levels of α-synuclein prior to aggregate formation (n = 9) compared with age- and post-mortem delay matched controls (n = 10). In the early stages of PD, mRNA expression of all LAMP2 isoforms was not different from control levels, with LAMP2B and LAMP2C protein levels also unchanged in PD. The selective loss of LAMP2A protein was directly related to the increased levels of α-synuclein and decreased levels of the CMA chaperone Hsc70 in the same PD samples, and resulted in the accumulation of the cytosolic CMA substrate proteins IκBα and MEF2D. These data show that LAMP2 protein isoforms are differentially affected in the early stages of PD, with LAMP2A selectively reduced in association with increased α-synuclein, and suggest that dysregulation of CMA-mediated protein degradation occurs prior to substantial α-synuclein aggregation in PD. (242 words)

POS-WED-058

SIMILAR DECREASES IN GLUCOCEREBROSIDASE LEVELS AND ACTIVITY ARE SEEN IN SPECIFIC BRAIN REGIONS IN PARKINSON'S DISEASE PATIENTS WITH AND WITHOUT *GBA1* MUTATIONS

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Heterozygous mutations in *GBA1*, encoding glucocerebrosidase (GCase), are the greatest genetic risk factor for Parkinson's disease (PD). Most homozygous patients with *GBA1* mutations and considerable GCase deficiency do not develop PD, suggesting heterozygous mutations may confer risk through a pathogenic gain-of-function associated with abnormal protein accumulation. To determine if there are any differences in abnormal protein accumulation and levels and activity of GCase in PD cases with and without *GBA1* mutations, cortical brain regions with (anterior cingulate) and without (occipital) a PD-related increase in abnormal α-synuclein protein levels were assessed in *GBA1*-mutant PD cases (n=10) and PD cases without *GBA1* mutations matched for disease severity (n=12). GCase and α-synuclein protein and mRNA levels were assessed by Western immunoblotting and qPCR, and GCase enzyme activity measured by fluorimetric assay. Compared to age-matched controls without disease or *GBA1* mutations (n=10), all PD cases had increased levels and aggregation of α-synuclein, and reduced GCase. GCase protein and enzyme activity were selectively reduced in PD anterior cingulate, while *GBA1* mRNA expression was reduced across both regions. GCase protein, enzyme activity and *GBA1* mRNA in *GBA1*-mutant PD anterior cingulate showed a small but non-significant reduction from PD cases without *GBA1* mutations. These data demonstrate that there is no significant difference in GCase deficits and associated α-synuclein pathology in PD cases with heterozygous *GBA1* mutations compared to those without, consistent with findings from recent studies[1,2]. This strengthens the suggestion that GCase deficiency rather than dysfunctional mutant GCase is most closely associated with α-synuclein accumulation in PD. (250 words)

[1]Parkkinen *et al.*, Mol Genet Metab 103:410-2

[2]Gegg *et al.*, Ann Neurol 72:455-63

POS-WED-059

THE EFFECTS OF MATERNAL IMMUNE ACTIVATION AND MK-801 ON MISMATCH RESPONSES IN AWAKE, FREELY-MOVING RATS

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Reduced mismatch negativity (MMN) to rare deviant sounds is one of the most robust schizophrenia endophenotypes and has been attributed to impaired NMDAR signalling. We investigated the effects of maternal immune activation (MIA; a model of schizophrenia) and pharmacological blockade of NMDAR on mismatch responses in rats. Electrodes were surgically implanted over five cortical locations in MIA and control male offspring and EEG was recorded during presentation of two oddball sequences (either a high- or low-frequency deviant), and a control sequence of randomly-intermixed frequencies that controlled for adaptation. Rats were tested off-drug, and after escalating doses of MK-801 (0.1, 0.3 and 0.5mg/kg). Five components of the ERP were identified: P13, N18, and P30, followed by a negative shift with two broad peaks, N-early and N-late. MIA and MK-801 increased deviance detection at P13, N18 and P30. Deviance detection evident in the N-early component was reduced by MK-801 but was unaffected by MIA. MIA increased deviance detection over the N-late range, which was normalised by MK-801. Deviance detection evident in the N-early component most closely resembles human MMN in terms of sensitivity to NMDAR antagonists. However, MIA in rats did not alter deviance detection of this component. The findings of *increased* deviance detection in MIA-exposed rats indicate that schizophrenia-related MMN impairments are not recapitulated in this model. However, for several components, the effects of MIA and MK-801 were similar and in some cases, potentiated each other, indicating that MIA and MK-801 may be converging on similar neurobiological systems.

(247)

POS-WED-060

COGNITIVE ABILITY IN THE ELDERLY IS RELATED TO THE TIMING NOT DEGREE OF LATERALISATION OF THE FUNCTIONAL CEREBROVASCULAR RESPONSE

Hofmann, J.[1], Flitton, A.[1], Kurylowicz, L.[1], Lavrencic, L.[1], Badcock, N.[2], Churches, O.F.[3], Kohler, M.[1], Keage, H.A.D.[1]

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Abstract

Objective. We investigated associations between cerebrovascular system functioning during a cognitive task and overall cognitive performance in healthy adults 60 years and over, using a cost-effective and well tolerated method – transcranial Doppler ultrasonography (TCD).
Methods. Twenty-five healthy right-handed individuals (17 female, 9 male) between the ages of 60 and 84 years underwent cognitive testing via the Weschler Abbreviated Intelligence Scale Vocabulary and Matrix sub-tests, and functional TCD testing during a word-generation task. TCD blood flow velocity data was collected from the right and left middle cerebral arteries. This data was averaged relative to stimulus onset (i.e. the letter required for the word generation task; 24 trials in total) and an evoked-flow plot for each individual was created.
Key Findings. The degree of lateralisation (i.e. left minus right middle cerebral artery blood flow velocities) during the word generation task was unrelated to overall cognitive ability. There was however a significant positive correlation between when peak lateralisation occurred (i.e. seconds post-stimulus) and cognitive ability. There was no relationship between resting blood flow velocity measures and cognitive ability, including the pulsatility index which is a measure of vessel resistance.
Conclusion. Those with higher cognitive ability in later adulthood appear to take longer to reach their 'peak lateralised state' than those with lower cognitive ability. The degree of cerebral lateralisation to a verbal task appears unrelated to cognitive performance. The timing of the cerebrovascular response during a cognitive operation may prove a useful marker of age-related cognitive decline.

POS-WED-061

SRC PROTEIN TYROSINE KINASE IS IMPORTANT FOR SURVIVAL OF CULTURED PRIMARY CORTICAL NEURONS

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Five members of Src family kinases (SFKs) namely Src, Fyn, Yes, Lyn and Lck are widely expressed in mammalian central nervous system. SFKs have been found to be involved in neuronal survival and neuronal cell death pathways; however the exact role of each of the members still remains unclear. SFK member Src kinase, is important for glial cell line-derived neurotrophic factor (GDNF) and thyroid hormone tri-iodothyronine (T3) mediated neurotrophic signalling pathways. Here we report the critical role of Src protein tyrosine kinase in supporting survival of cultured primary cortical neurons. Treatment with specific SFK inhibitor (SU6656) significantly reduced cell viability of cultured primary cortical neurons at day 7 (DIV7). Similar phenomenon was observed when neuronal Src was specifically knocked-down using specific short-hairpin RNA (shRNA), demonstrating its indispensable role in survival of primary cortical neurons. Both SFK inhibitor treatment and specific knock-down of Src expression was found to be associated with inhibition of Akt phosphorylation. Our data suggest that Src activity is crucial for neuronal cell survival and this activity is somewhat relate to neurotrophic Akt signalling pathway. (177 words)

POS-WED-062

DETERMINING THE SUB-CELLULAR LOCALISATION OF CU^{II}(ATSM), A NEUROPROTECTIVE THERAPEUTIC IN MODELS OFAMYOTROPHIC LATERAL SCLEROSIS.

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Patients with Amyotrophic Lateral Sclerosis (ALS), the most common form of Motor Neuron Disease, have limited therapeutics available. Recently, it has been shown that the drug Cu^{II}(atsm) is neuroprotective in multiple mouse models of ALS, however the exact mechanism of neuroprotection remains unknown. This study aims to provide information on how this therapeutic is neuroprotective, by determining the sub-cellular localisation of Cu^{II}(atsm). This study investigated the sub-cellular localisation of Cu^{II}(atsm) in both M17 neuroblastoma secondary cells and murine primary cortical neurons. The therapeutic Cu^{II}(atsm) was conjugated to a bodipy fluorophore; live cells were treated with the bodipy bound Cu^{II}(atsm) and imaged via confocal imaging. It was found that Cu^{II}(atsm) is largely cytosolic, and that that there is partial lysosomal colocalisation with Cu^{II}(atsm). X-ray fluorescence, done at the Australian Synchrotron, showed an increase in copper levels after treatment with Cu^{II}(atsm). Cells treated in absence of Cu^{II}(atsm) had minimal copper levels, however those treated with Cu^{II}(atsm) had a much greater copper content ($n \geq 3$, $P<0.05$), consistent with uptake of this therapeutic.

This study importantly shows that the therapeutic Cu^{II}(atsm) is readily uptaken in neuronal cells, and has a cytosolic distribution, with partial lysosome accumulation. (191 words)

POS-WED-063

CHOLESTEROL METABOLISM IS DISTURBED EARLY IN HUMAN ALZHEIMER'S DISEASE BRAIN

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Cholesterol is a highly abundant lipid in the brain. Its synthesis and metabolism is tightly regulated to maintain normal neurological function. Cholesterol metabolism is known to be disturbed in multiple neurodegenerative diseases. This study measured sterol changes (including cholesterol metabolites and synthetic precursors) in human Alzheimer's disease (AD) post mortem tissue to identify potential biomarkers of AD progression.

Lipid was extracted from hippocampus (HC) and cerebellum (CB) and analysed using gas chromatography-mass spectrometry. Sterol levels were compared to the clinical severity of AD (n= 9 control and n=25 Braak stage I-VI).

The major brain cholesterol metabolite 24-hydroxycholesterol (24-OHC) formed by the neuronal specific enzyme CYP46A1, was significantly reduced in HC at later AD stages ($p>0.05$), but was unaffected in CB. At later AD stages in HC, 27-hydroxycholesterol was significant increased, but not in CB. Interestingly in both brain regions we detected significant decreases in phytosterols (dietary derived) and the cholesterol synthetic precursor 24,25-dihydrolanosterol (DHL) ($p>0.05$).

Our data illustrates that the cholesterol CYP46A1 metabolic pathway is progressively disturbed during AD in HC, where the most severe atrophy occurs. This confirms previous studies of AD patients that detected altered 24-OHC plasma levels. Likewise, reduced DHL and phytosterols supports previous reports of reduced cholesterol synthetic precursors and phytosterols in CSF and plasma of AD patients. Our results indicate that altered brain sterols are an early pathological event in AD. Elucidating pathophysiological changes in brain cholesterol provides potential biomarkers for examining neurodegenerative disease and offers new targets for therapeutic intervention. (246 words)

POS-WED-064

REGULATION OF CATECHOLAMINE SYNTHETIC ENZYMES IN PARKINSON'S DISEASE: OBSERVATIONS FROM RODENT MODELS

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Parkinson's disease (PD) is characterised by progressive selective loss of catecholamine-producing cells in the brain and periphery. Currently there is no perfect animal model hindering the development of novel treatments. Our study combines rotenone and lipopolysaccharide (LPS), both used separately to model PD, to assess motor symptoms and investigate regulation of catecholamine synthetic enzymes, tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH), in rat brain and adrenal glands. Rats underwent treatments with low dose rotenone (2mg/kg, 5 days/week, n=5), LPS (200µg/kg, 1 day/week, n=4), rotenone + LPS (n=5) or vehicle (n=4) for 4 weeks. Parkinsonian motor deficits were assessed via the rearing test throughout treatment. Adrenal glands and brain regions known to be affected in PD were collected and analysed using the western blotting technique. A significant decrease in rearing was observed for rotenone ($p<0.0001$) and rotenone + LPS ($p<0.001$) groups vs control. LPS significantly increased pSer19TH ($p<0.05$), pSer31TH ($p<0.05$) and pSer40TH ($p<0.01$) vs. control in the substantia nigra; however, there was no significant difference in TH levels between any treatment groups in this region. TH and DBH were significantly decreased in LPS ($p<0.05$) and rotenone ($p<0.05$) groups in the olfactory bulb. Rotenone + LPS increased TH ($p<0.05$) and LPS increased pSer19TH ($p<0.05$) in the adrenal gland, illustrating the periphery is affected. The novel findings of decreased TH and DBH levels in the olfactory bulb without changes in the substantia nigra suggest that the low dose rotenone and LPS paradigms have potential for use to investigate early changes in PD. (words 249).

POS-WED-065

OPTIMISATION OF PHOTOTHROMBOTIC STROKE FOR PRODUCING LONG-TERM FUNCTIONAL DEFICITS

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Investigation of strategies that can promote recovery following cerebral ischaemia has been somewhat hampered by the establishment of robust models of long-term functional deficits. In this project we wished to develop a model of cerebral vascular occlusion and to establish a reliable set of neurobehavioural assessments that could readily identify persistent functional deficits. To introduce vascular occlusion we chose a photothrombotic occlusion procedure. In brief, this involves the injection of a photoactive dye that upon exposure to light initiates a clotting cascade, and subsequent vascular occlusion. Despite using a 'cold' light source we identified that high light intensity could result in significant heating of the cortical surface and induce damage in sham (no dye) animals. We further identified two tasks that were highly sensitive to identifying motor deficits, namely the cylinder task in the grid walk task.
Importantly, the dimensions of the grid square were observed to be a critical feature of the test sensitivity with 25 mm grid squares yielding significantly better results than other grid sizes evaluated. Using these optimised assessments we identified that photothrombotic occlusion of the motor cortex induced significant functional impairments that persisted until at least day 28. Interestingly, we observed a significant level of spontaneous recovery across the 28 day observation window, with significantly higher deficits occurring two days post stroke than compared to all other time points. In summary, we have established a model of cortical vascular occlusion that can be successfully deployed to investigate interventions that may improve recovery following cortical ischemia.

POS-WED-066

ORBITAL FRONTAL CORTEX LAMINAR DEFICITS IN SOMATOSTATIN AND PARVALBUMIN EXPRESSION ARE RELATED TO INCREASED WHITE MATTER NEURON DENSITY AND DEATH RECEPTORS IN SCHIZOPHRENIA

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*S*tructural abnormalities in the orbital frontal cortex (OFC) have been related to social-emotional deficits in schizophrenia, but little is known about the underlying cellular neuropathology. Recently, we provided neurobiological evidence showing inhibitory interneuron deficits in the OFC in schizophrenia. Here we determine the nature and extent of inhibitory interneuron deficit in the OFC in schizophrenia. We examine how laminar deficits in somatostatin (SST) and parvalbumin (PV) are related to gray matter gene expressions (Dlx1, GAD67, FAS receptor) and interstitial white matter neuron (IWMN) density in OFC. Laminar SST and PV mRNA expressions were determined in control (n=40) and schizophrenia (n=38) subjects using in situ hybridisation. SST and PV laminar mRNA levels were correlated with IWMN (GAD65/67, NeuN) density and with Dlx1, GAD67 and FASR mRNA levels measured by qPCR. In schizophrenia, SST mRNA level was reduced in layers I-VI, with highest deficits in layers II (66%, $p=1.91x10^{-19}$) and V (45%, $p=5.72x10^{-6}$), and increased in white matter. PV mRNA level was reduced in layer IV (31%, $p=1.10x10^{-4}$) in schizophrenia. SST mRNA level (layers I-VI) correlated positively with GAD67 but negatively with IWMN density and FASR. PV mRNA expression (layers I-VI) correlated positively with GAD67 and Dlx1 mRNAs. We provide the first evidence of prominent laminar deficits in SST and PV expressions in the OFC in schizophrenia. The negative correlations between SST/GAD67 mRNAs and cell death receptor mRNAs implicate cell death pathways in the interneuron deficit. Current findings advance our understanding of the interneuron pathology in the OFC in schizophrenia. **(248 words)**

Keywords: cell death receptors, interneuron, messenger RNA, orbital frontal cortex, schizophrenia

POS-WED-067

NERVE INJURY INCREASES SEROTONIN AND ITS METABOLITES IN THE MEDIAL PREFRONTAL CORTEX IN RATS WITH COMORBID SOCIAL BEHAVIOURAL DISTURBANCES

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Medial prefrontal cortex (mPFC) exerts executive regulation of emotional coping responses; its extensive limbic, diencephalic and brainstem outputs regulate the behavioural, neuroendocrine and autonomic components of these responses. mPFC function is regulated by catecholaminergic (CA) and serotonergic (5-HT) inputs, and also by Brain Derived Neurotrophic Factor (BDNF). There are reciprocal relationships between BDNF expression and CA and 5-HT release. Disruptions to these relationships result in dysfunctional coping responses. In a subset of rats, nerve injury triggers altered coping, identified by disrupted social behaviours. We investigated the relationship between BDNF, dopamine (DA), noradrenaline (NE) and 5-HT in the mPFC of rats, with disrupted social interactions following sciatic nerve injury (CCI). Rats received either CCI (N=13) or sham injury (N=5), social behavior was quantified for six days before and after the surgery, rats with altered social dominance were identified. HPLC was used to determine DA, 5HT, NE and their metabolites in the mPFC of all rats. BDNF levels were determined using ELISA. Injury triggered reductions in dominance correlated with increased 5-HT (r^2=0.344, p=0.03) and it metabolite 5-HIAA (r^2=0.356, p=0.03) in the mPFC contralateral to the nerve injury. The levels of BDNF protein in the contralateral mPFC were positively correlated with the 5-HT levels determined by HPLC (r^2=0.552, p<0.001). These data raise the possibility that in a select group of animals, CCI triggers increases in 5-HT and BDNF in the mPFC, which alters the executive regulation of coping behaviours resulting in the disrupted social behaviours, which characterize this subpopulation of rats.

POS-WED-068

POTENCY OF A MUSCLE-DERIVED ISOFORM OF IGF-1 FOR THE RESCUE FACIAL MOTONEURONES AND INSIGHT INTO ITS MECHANISM OF ACTION

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We previously found that gene transfer of muscle-derived isoform of Insulin-like Growth Factor-1 (IGF-1) termed Mechano-Growth Factor (MGF) rescued 80% of avulsed facial motoneurones when 80% would have died. Little, however, is known about MGF's receptor and its mechanism of action. Here we examined whether MGF neuroprotection is mediated via the IGF-1 receptor and activation of protein kinase C (PKC). The right facial nerve was avulsed at the stylomastoid foramen (SMF) in 6 anaesthetised adult Sprague-Dawley rats. In four further groups of (n=5-6), avulsion was followed by injection into the SMF of 10µl of saline or MGF, IGF-1 or Glial-cell-line Derived Neurotrophic Factor (GDNF) at 1µg/µl. In two other groups (n=5-6), MGF was co-injected with 1µg/µl of either (i) an IGF-1 receptor antibody, or (ii) the PKC inhibitor GF109203X. Two further groups (n=4-9) received IGF-1 or GDNF co-administered with antisera to their respective receptors. Rats were perfusion-fixed 1 month later and numbers of motoneurones determined stereologically.1 month following avulsion only and avulsion plus saline, 80% of motoneurones were lost. This loss was reduced to 50%, 20% and 10% by MGF, GDNF and IGF-1, respectively ($p<0.05$ vs. avulsion only). Injection of IGF-1 or GDNF with their receptor antagonists resulted in motoneuronal losses of 44% and 50%, respectively. Co-injection of MGF with GF109203X or antisera to the IGF-1 receptor had no effect on motoneurone loss compared to MGF alone. This indicates that motoneuronal rescue by the MGF peptide may be independent of both the IGF-1 receptor and PKC activation. (247 words)

POS-WED-069

TDP-43 PATHOLOGY IN A UK POPULATION-BASED COHORT: PREVALENCE AND ASSOCIATIONS WITH DEMENTIA AND AGE

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Objective. To aimed to determine the prevalence of transactive response DNA-binding protein of 43 kDA (TDP-43) neuronal inclusions in a population-based sample, and associations with age group at death (≤90 and >90 years) and clinical dementia status prior to death. Further, to investigate associations between TDP-43 inclusions and other key dementia-related neuropathologies (plaques, tangles and neuronal loss) within the hippocampus, entorhinal and temporal cortices.
Methods. All brain donors within the Cambridge City over-75s Cohort (CC75C), which is population-based and longitudinally tracked (n=228) were included. Age at death ranged from 78 to 106 years. TDP-43 neuronal inclusions were assessed in the hippocampus, entorhinal cortex and temporal cortex. These data were combined with existing clinical and neuropathological data.
Key Findings. TDP-43 neuronal inclusions were present in 27% of the sample - 36% of those with clinical dementia and 18% without dementia. Individuals who died later (>90 years) or with clinical dementia were more likely to show TDP-43 inclusions. Hippocampal and entorhinal TDP-43 inclusions were significantly associated with dementia severity and increasing age, taking into account other neuropathologies. TDP-43 neuronal inclusions appeared to co-localised with severe neuronal loss.
Conclusion. Findings indicate that hippocampal and entorhinal TDP-43 inclusions are key substrates of late onset dementia which appear to co-localise with severe neuronal loss, but not with Alzheimer's markers of amyloid and tau. This broadens the accepted view of TDP-43 pathology in dementias and contributes to the understanding of the underlying biology of dementia in the very old.

POS-WED-070

MEASUREMENT OF LRRK2 IN PERIPHERAL IMMUNE CELLS.

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There is currently much interest in the leucine-rich repeat kinase 2 (LRRK2) as a potential therapeutic target for the treatment of Parkinson's disease (PD). Mutations in LRRK2 cause autosomal-dominant PD and the most common mutation (G2019S), increases the enzymes kinase activity. As a result a number of LRRK2 kinase inhibitors have been developed. We have previously shown in cell and animal models that LRRK2 kinase inhibitors cause a dose-dependent decrease in the phosphorylation of two residues on LRRK2, S910 and S935. These residues, which are not autophosphorylation sites, regulate the binding of LRRK2 to 14-3-3 adaptor proteins. Moreover we have also demonstrated using mouse macrophages that the phosphorylation of S910 and S935 increases with stimulation of certain inflammatory pathways. In the present study we have measured LRRK2 protein and its phosphorylation at S910 and S935 in PBMCs from control and Parkinson's disease patients. We found that peripheral LRRK2 has high intrinsic phosphorylation that does not associate with PD or PD-relevant measures. Treatment of PBMCs with inflammatory agonists could not increase LRRK2 S910 or S935 phosphorylation further. However, LRRK2 kinase inhibitors could dose-dependently decrease LRRK2 phosphorylation at S910 and S935 in human PBMCs. These findings could be exploited to develop a convenient pharmacodynamic readout for LRRK2 inhibitors. (206 words).

POS-WED-071

TOOTH STROKE STUDY – THE OPEN STUDY OF DENTAL PULP STEM CELL THERAPY IN HUMANS.

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Stroke is the leading cause of disability in Australia with over 300,000 people currently living with this challenge to their quality of life. In 2013 the few evidence-based treatments in stroke include: aspirin, thrombolysis, management in a stroke unit and hemicraniectomy. The challenge in neuroscience is how to repair the brain following injury from stroke and other insults. One potential approach is cell-based therapy. We have spent the last decade investigating one stem cell type isolated from the human tooth – dental pulp stem cell (DPSC). This is a clinically accessible source of potential autologous human adult stem cells with neurogenic potential (Arthur et al, 2008; 2009). We were one of the first to demonstrate that DPSC therapy in a rodent stroke model resulted in enhanced neurological recovery (Leong et al., 2012). We have systematically meta-analyzed a decade of data using varied stem cell types to treat the rodent stroke model and overall there is approximately 40% improvement in neurological function (Lees et al., 2012). There is controversy as to when to translate from pre-clinical studies to clinical trials in cell-based therapy in stroke. We propose the TOOTH Stroke Study that will investigate the safety and feasibility of autologous DPSC transplantation in humans who have chronic disability following stroke. We will present the protocol of this Phase 1 clinical trial and describe the hurdles that lay ahead to undertake such a trial in Australia (233 words).

POS-WED-072

ALTERED CHOLESTEROL METABOLISM AND INCREASED CHOLESTEROL PEROXIDATION IN HUMAN POST MORTEM HUNTINGTON'S DISEASE BRAIN

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Cholesterol is a highly abundant lipid in the brain. Its synthesis and metabolism is tightly regulated to maintain normal neurological function. Cholesterol metabolism is known to be disturbed in multiple neurodegenerative diseases. This study aimed to measure changes in cholesterol metabolites and cholesterol oxidation products (COPs - lipid damage biomarkers), in human Huntington's disease post mortem tissue and identify potential biomarkers of the disease.

Lipid was extracted from post mortem brain tissue (obtained from the ABBN) and analysed using gas chromatography-mass spectrometry. Five brain regions of HD (≥ stage 3) and age matched controls were analysed; caudate, putamen, cerebellum, grey and white matter frontal cortex (n=9 per group).

The major brain cholesterol metabolite 24-hydroxycholesterol (24-OHC) formed by the neuronal specific enzyme CYP46A1, was significantly reduced in HD putamen ($p<0.0001$), caudate ($p=0.0109$) and white matter ($p=0.0448$). No significant reductions were observed in cerebellum and grey matter. 7-Ketocholesterol (COP) was significantly increased in HD putamen ($p=0.0065$). Small increases in 7β-OH cholesterol (COP) were also detected in caudate and putamen, however these were not statistically significant.

Our data illustrates that the cholesterol CYP46A1 metabolic pathway is disturbed in HD brain, particularly the striatum (caudate and putamen) where the most severe atrophy occurs. This confirms previous studies of HD patients that detect decreases in 24-OHC plasma levels. Oxidative stress is also evident in multiple HD brain regions, particularly putamen. Elucidating pathophysiological changes in brain cholesterol provides potential biomarkers for examining neurodegenerative disease and offers new targets for therapeutic intervention. (245 words)

POS-WED-073

SLEEP ELECTROENCEPHALOGRAPHIC SPECTRAL POWER IN ALCOHOL-DEPENDENT PATIENTS

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Objectives: Sleep problems, which can have significant clinical consequences, are more common among alcohol dependent patients than among normal healthy subjects. The gold standard for objectively measuring sleep is polysomnography. In the present study, we used spectral power analysis of the sleep electroencephalographic (EEG) to quantify brain activity during sleep in patients as well as in control subjects.

Methods: 20 patients with alcohol dependence syndrome diagnosed as per ICD-10 (DCR), who have completed three weeks of detoxification in hospital, and meeting the exclusion-inclusion criteria were initially enrolled for the study. Patients were rated on HDRS, HAM-A, ASI, GAF, Sidedness Bias Schedule and SDQ at the time of enrollment. 20 normal healthy controls matched in terms of age and sex fulfilling inclusion-exclusion criteria were rated on Sidedness Bias Schedule, SDQ and GHQ-12. Throughout the night 40 channels polysomnographic recording was done for each of the patient and normal control for a single night using the Sandman Elite 7.2.1 software. Scoring for sleep stages and other events was done using Rechtshaffen and Kales criteria. Both computers assisted and manual scoring was done. 60 seconds epoch from each of the stages (NREM Stage 2, 3 and 4 and REM) of each subject were taken for computing power spectrum using the software Matlab 6.5.

Results: Patients had increased power in beta and gamma 1 bands in all regions during REM sleep as compared to controls. Controls showed increased power in delta band in the left temporal and right parietal, temporal, occipital and central region as compare to patient group during stage 2 and stage 3 sleeps..

Conclusion: Patients had increased power in beta and gamma 1 bands in all regions during REM sleep which could be interpreted as a sign of dysfunctional arousal during REM sleep.

POS-WED-074

MODULATION OF MIDBRAIN DOPAMINERGIC AND NEUROTROPHIC PATHWAYS BY TESTOSTERONE

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Risk for the dopamine-associated disorders Parkinson's disease and schizophrenia varies with age and sex. This is thought to be partly attributable to gender- and age-related variations in circulating sex steroid levels. While oestrogens are neuroprotective, the role of testosterone is controversial. We hypothesised that testosterone enhances glial cell line-derived neurotrophic factor (GDNF; a potent dopaminergic neurotrophic factor) signalling mechanisms and dopamine synthesis pathways in male substantia nigra. Male rats at 45 days of age (adolescence) and at 22-24 months of age (senescence) were left intact or gonadectomised, followed by vehicle or testosterone, dihydrotestosterone or 17*β*-oestradiol replacement via subcutaneous implants for two weeks (*n*=15/group). Quantitative real-time PCR and immunoblotting were performed to quantify mRNA and protein expression of GDNF and GDNF receptors (GFRα1 and RET); tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis; and phosphorylated TH (TH-P), the active form of TH. Adolescent and aged GDNF levels were unchanged by sex steroid modification. Testosterone removal decreased adolescent GFRα1 mRNA expression by 15%, which was restored by androgen treatment. We have previously shown that testosterone increases TH protein in the adolescent male rat substantia nigra. By contrast, in the aged cohort, gonadectomy reduced TH-P protein by 53.0%, which was restored by dihydrotestosterone, but not by testosterone. These data support the hypothesis that testosterone, via androgenic activation, modulates dopamine synthesis and GDNF signalling pathways. The effect and mechanism of this modulation, however, may vary with age in a manner that reflects differential male vulnerability to dopaminergic diseases at different life stages. (249 words)

POS-WED-075

UNDERSTANDING THE ROLE OF REGULATORY T CELLS IN CHRONIC PAIN FOLLOWING NERVE INJURY

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Neuropathic pain is a debilitating condition caused by damage to the somatosensory nervous system, such as peripheral nerve injury. The immune system plays a key role in mediating such pain. Regulatory T (Treg) cells are a small subpopulation of T cells with immunosuppressive function. Here, we investigated the effects of depletion of Treg cells on mechanical allodynia in mice with chronic constriction injury (CCI) of the sciatic nerve. We detected small numbers of Treg cells expressing FoxP3 by immuno-fluorescent staining in the sciatic nerve, dorsal root ganglia and spinal cord of CCI injured-mice, but not in sham control mice. We then utilised a transgenic mouse model (DEREG – DEpletion of REGulatory T cells) that expresses green fluorescent protein (GFP) tagged human diphtheria toxin (DT) receptor under the control of the FoxP3 promoter (Treg marker). Flow cytometric analysis of GFP revealed a 10 fold reduction in the CD4+GFP+ cell compartment from the inguinal lymph node of DT-treated DEREG mice, as compared to vehicle-treated DEREG mice, indicating FoxP3+ Tregs depletion. Following CCI, we observed a decrease in pain thresholds of Treg-depleted DEREG mice compared to control groups that corresponded to the timing of Treg depletion. Analysis of cytokines present within the serum taken from these mice showed significant systemic changes in cytokine expression profiles, which may also act to regulate neuropathic pain. These results indicate that depleting Treg cells increases pain hypersensitivity suggesting that Treg cells act to suppress neuropathic pain. Thus, Treg cells might present a possible therapeutic approach to neuropathic pain.

POS-WED-076

DIFFERENCES IN OREXIN GENE REGULATION AND PEPTIDE EXPRESSION IN THE HYPOTHALAMUS OF NERVE-INJURED RATS WITH AND WITHOUT CO-MORBID DISRUPTIONS IN SOCIAL BEHAVIOUR

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Objective: Nerve injury evokes a state of neuropathic pain, with comorbid changes in weight gain and social behaviours, in only some people. The regulation of body weight and aspects of social behaviour depends on peptidergic circuitry of the hypothalamus, including orexigenic pathways. We sought to determine the effects of nerve injury on these circuits and their relationship to weight gain and social behaviours in rats. **Methods:** Rats underwent sciatic nerve constriction injury [CCI] (N=32) or sham injury (N=12) and their weight and social behaviours were compared for six days before and after injury. RT-PCR for NPY, AGRP, orexin, CART and alpha-MSH mRNAs was performed in isolated hypothalamic blocks (right & left). On the basis of this analysis, standard immunohistochemical procedures were used to probe serial sections of hypothalamus, from 18 CCI and 6 sham rats, to determine the number, location and intensity of orexin-immunoreactive (-IR) neurons. **Results:** RT-PCR revealed a bilateral, two-fold down-regulation of orexin mRNA in CCI rats compared to shams. Orexin cell numbers in lateral hypothalamus (LH) were negatively correlated with weight gain.
Reductions in the expression of social dominance in CCI rats correlated with greater numbers of orexin-IR cells in the LH, medial to the fornix. Rats whose social behaviours were unaffected by CCI had the least numbers of orexin-IR cells in the perifornical LH. **Conclusion:** We have revealed evidence of regional relationships between the expression of orexin in lateral hypothalamic subregions with both weight gain and social behavioural changes triggered in subsets of rats following CCI. (250 Words)

POS-WED-077

NEURON-SPECIFIC EXPRESSION OF HUMAN APOLIPOPROTEIN D ALLEVIATES COGNITIVE IMPAIRMENTS AND REDUCES AMYLOID DEPOSITION IN APP/PS1 ALZHEIMER'S DISEASE MICE.

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In the adult brain neurons do not appear to synthesise apolipoprotein D (apoD), however, apoD mRNA and/or protein has been detected in apparently healthy neurons in human Alzheimer's disease (AD) tissues and in aged mouse brain. This might suggest that apoD has a neuroprotective role in AD. To assess this we crossed human apoD transgenic mice (selectively expressing human apoD in neurons via the Thy-1 promoter) with APP/PS1 AD mice to generate APP/PS1-ApoDtg mice and APP/PS1-WT littermates. At ~ 10 months of age we used the Cheeseboard paradigm (to find a food reward) to assess cognitive capacity, and at ~12 months of age AD-related pathology was subsequently assessed. In a first test cohort, we found an increase in the proportion of time APP/PS1-ApoDtg mice spent in the target zone in the Cheeseboard test as compared to APP/PS1-WT mice. Histological analysis revealed that hippocampal amyloid plaque load was significantly reduced by ~30% (<0.05) in the APP/PS1-ApoDtg mice compared to the APP/PS1-WT mice (using either 6E10 immunohistochemisty or Thioflavine-S staining). We also detected a significant decrease in guanidine HCl-soluble amyloid β (Aβ) 40 (35%, P<0.05) and Aβ42 34%, (P<0.05) in hippocampal homogenates analysed by ELISA. Interestingly, significant reductions of amyloid precursor protein-C-terminal fragments APP-CTFs (CTF-α, CTF-β, and AICD) were also detected (all ~45% reduced, P<0.05). Our preliminary data provides evidence that neuronal apoD expression may confer protection in AD setting. Furthermore, therapeutic approaches to increase apoD expression may represent a novel avenue to retard AD progression. (244 words)

POS-WED-078

MITOCHONDRIA-ER CONTACT LENGTHS IN ZEBRAFISH EMBRYOS WITH INHIBITION OF PRESENILIN1 AND PRESENILIN2 GENE EXPRESSION.

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Mutations in the genes PRESENILIN1 (PSEN1), PRESENILIN2 (PSEN2) and APP have been identified in familial Alzheimer's disease (AD). Area-Gomez et al. (2012) EMBO J. 31:4106-23 showed that the length of mitochondria-ER contacts is increased in Psen1-/-/Psen2-/- double knockout murine embryonic fibroblasts and in AD patient fibroblasts. To determine the effect of inhibition of PSEN1 and PSEN2 activity on mitochondria-ER contacts in the zebrafish animal model, we injected zebrafish embryos with morpholinos (MOs) that inhibit expression of zebrafish psen1 and psen2. We then analysed mitochondria-ER apposition in neural cells under electron microscopy. Our analysis showed no significant difference in mitochondria-ER contact lengths between control MO and PSEN1 & PSEN2 morpholino co-injected embryos at 24 hours post fertilization. Instead, the distribution of mitochondria-ER apposition lengths into different length classes was close to identical (total apposition events analysed was 23 in control and 25 in MO-injected embryos). While our observations differ from those of the above cited study, this may be due to differences in cell type, cell age, efficiency of PRESENILIN gene functional inhibition and differences in organism species. (214 words)

POS-WED-079

CO-TRANSPLANTATION OF NEURAL STEM CELLS AND OLFACTORY ENSHEATHING CELLS PROMOTES NEUROLOGICAL RECOVERY ASSOCIATED WITH IGF1 UP-REGULATION AFTER COMPLETE SPINAL CORD TRANSECTION

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Combined neural stem cells (NSCs) and olfactory ensheathing cells (OECs) transplantation may become an optimal strategy for the treatment of spinal cord injury (SCI). To maximize the differentiation of NSCs into neurons and guide the axonal regeneration through the astroglial scar is the key problem in the treatment of central nervous system (CNS) injury. Gelatin scaffold may be a good candidate to provide a suitable niche. This study determine the synergistic effect and underlying molecular mechanism of combined transplantation of NSCs and OECs in gelatin scaffolds in spinal cord transection adult rats. We found that co-transplanted of NSCs and OECs in gelatin scaffold into the transected spinal cord (T10) of rats significantly increased Basso, Beattie, and Bresnahan (BBB) scores, ameliorated the latency of cortical somatosensory evoked potential (CSEP) responses and promoted axons regeneration. Combined transplantation regenerated more axons and increased the neuronal number at caudal cord than in NSCs or OECs alone group. Moreover, the insulin-like growth factor 1 (IGF1) was significantly increased in co-transplanted group compared with NSCs or OECs group, respectively. These results indicated that gelatin scaffold may provide a suitable local environment for SCI repair. Co-transplantation of NSCs and OECs, as a therapeutic intervention for the treatment of SCI, might improve locomotor recovery via increasing IGF1 expression.

POS-WED-080

BEHAVIOURAL RESPONSES TO A 5-HT$_{1B}$ AGONIST FOLLOWING MDMA SELF-ADMINISTRATION

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A significant body of research has suggested a role of the serotonin 1B (5-HT$_{1B}$) receptor in drug reinforcement because of its ability to modulate dopamine (DA) neurotransmission. 5-HT$_{1B}$ agonists increase DA release via activation of heteroreceptors localized on GABA and Glutamate afferents. Because MDMA preferentially stimulates the release of 5-HT, the possibility that repeated exposure might impact these receptors was investigated. The effect of MDMA self-administration on the behavioural responses to the 5-HT$_{1A/1B}$ agonist, RU 24969, was determined and compared to rats that had self-administered vehicle. Rats self-administered a total of 350 mg/kg of MDMA during 22-75 daily 2 hour sessions. The day following the last self-administration session, the effect of RU 24949 (0.03-3.0 mg/kg) on hyperactivity and fluid consumption was measured. RU 24969 dose-dependently increased locomotor activity and decreased fluid consumption in control rats. The dose-effect curve was shifted to the right in rats that had self-administered MDMA. These results suggest a desensitization of the 5-HT$_{1B}$ receptor following MDMA self-administration. (161 words).

POS-WED-081

PERCEPTUAL LEARNING OF TACTILE LETTERS TRANSFERS ACROSS BODY SURFACES

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Visual-to-tactile sensory substitution devices aim at assisting visually impaired people by converting visual stimuli into tactile stimuli. Studies conducted with these devices revealed an important structural and functional plasticity of the central nervous system. The important claim has been made that, after training, the tactile stimuli can be moved from one body surface to another without decrease in performance. This claim, although recurrent, has never been empirically investigated. Moreover, studies in the field of tactile perceptual learning suggest that performance improvement transfer only to body surfaces that are closely represented in the somatosensory cortex. These studies have however mainly used discrimination tasks of stimuli varying along only one feature (e.g., orientation of gratings) whereas, in sensory substitution, tactile information consists in more complex stimuli. The present study investigated the extent to which there is a transfer of tactile letters learning. Participants first underwent a baseline session in which the letters were presented on their belly, thigh, and shin. They were subsequently trained on only one of these body surfaces, and then re-tested on all of them, as a post-training session. The results revealed a significant performance improvement that was the same for both the trained and the untrained surfaces. Moreover, this transfer of perceptual learning was equivalent for adjacent and non-adjacent body surfaces, suggesting that tactile learning transfers independently of the topographic organization of the somatosensory cortex. The obtained results support the claim that training with sensory substitution devices results in a relative independence from the stimulated body surface. (249)

POS-WED-082

IDENTIFICATION OF TYPE I POSITIVE ALLOSTERIC MODULATORS OF α7 NICOTINIC ACETYL CHOLINE RECEPTOR FOR THE TREATMENT OF COGNITIVE IMPAIRMENT IN NEURODEGENERATIVE AND PSYCHIATRIC DISEASE.

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Positive allosteric modulation (PAM) of the α7 nicotinic acetylcholine receptor (α7 nAChR) offers a promising therapeutic strategy for cognitive enhancement in disorders including Alzheimer's disease, Parkinson's disease, schizophrenia and ADHD. We sought to characterise a selection of PAMs of α7 nAChR utilising electrophysiology and to develop simple metrics for type I and type II PAM identification. Electrophysiology was used in conjunction with animal models of cognition to correlate *in vitro* potentiation with *in vivo* effect. This approach underpinned our medicinal chemistry campaign to develop novel type I PAMs. Early efforts led to the identification of compounds BL-009782 and BL-009796, which were characterised as type I PAMs by electrophysiology. Both BL-009782 and BL-009796 exhibited pro-cognitive effects in the mouse T-maze continuous alternation task (T-CAT). Advancement of the chemical series to improve the pharmacokinetic profile of the compounds generated compound BL-010343, a strongly potentiating type I PAM, which was selected for broader profiling. (151 words).

POS-WED-083

PHYSICAL EXERCISE IMPACTS ON COGNITIVE, AND DEPRESSIVE-LIKE BEHAVIOURS IN C57BL/6 MICE.

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Exercise is known to result in beneficial effects on cognition and aspects of mood including anxiety and depression however the effects of varied parameters of exercise on these factors are less well understood. The objective of this study was to investigate the effects of voluntary exercise on indicators of cognition, anxiety and mood in rodent early adulthood. Twenty two 12 week old C57BL/6 mice were randomised to either control (n=11) or voluntary wheel running exercise (n=11) groups for four weeks. Behavioural testing was then undertaken to assess cognition, anxiety and depression-like behaviour. The Barnes maze was used to test cognition, anxiety-like behaviour was assessed with the Elevated Zero Maze, and depression-like behaviour was measured using the Forced Swim Test. We found that in the Barnes maze, both groups of mice showed learning over 4 days of training. However exercised mice demonstrated significantly higher latencies to find the escape box on all days, and a higher spatial retention memory during a probe trial, suggesting impaired cognition. Exercised mice also demonstrated reduced time in open arms of the Elevated Zero Maze and greater immobility time in the Forced Swim Test, indicating greater anxiety-like and depression-like behaviours. Exercise did not result in the expected improvements in cognition, anxiety, or depression-like behaviours in young adult rodents. Conversely, exercise seems to have impaired these behaviours in this cohort. These findings highlight the need for greater understanding of the effects of varied exercise parameters on cognition, anxiety and mood at different stages of the lifespan.

(Word count : 237)

POS-WED-084

DYSEXECUTIVE SYNDROME: FRONTO-STRIATAL DISCONNECTION AND DISORDERS OF DECISION- MAKING.

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The fronto-striatal pathway has long been known to play an important role in executive functions, particularly in decision-making involving goal-directed actions, and changes in this pathway have been linked to the cognitive symptoms associated with various forms of psychiatric disorder, neurodegenerative conditions and addiction. Recent research in animal models has established that this pathway is essential for striatal plasticity associated with the acquisition of new actions; damage to this pathway renders actions less deliberated and more impulsive or habitual. By examining pathway-specific activity using tract tracing coupled with immunohistochemistry, we also found direct evidence that a circuit linking prelimbic prefrontal cortex and a posterior region of dorsomedial striatum mediates the acquisition and consolidation of goal-directed actions. Although this pathway is not required during retrieval, the neurons targeted by this pathway are essential for action selection and evaluation in decision-making.

POS-WED-085

OXYTOCIN ADMINISTRATION IN THE NUCLEUS ACCUMBENS CORE OF THE RAT REDUCES REINSTATEMENT OF METHAMPHETAMINE-SEEKING.

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The psychostimulant methamphetamine (METH) is an addictive drug of abuse. The neuropeptide oxytocin has been shown to modulate METH-related reward and METH-seeking behaviour. Recent findings implicated the nucleus accumbens core (NAcc) as a key substrate in oxytocin modulation of METH-induced reward. It is not known, however, if oxytocin acts in this region to reduce relapse to METH-seeking behaviour, and if this action is through the oxytocin receptor. Using the reinstatement paradigm in rats experienced at METH self-administration, we aimed to determine whether oxytocin pretreatment within the NAcc would reduce relapse to METH use and if this could be reversed by the co-administration of the oxytocin antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT. Male Sprague Dawley rats underwent surgery to implant an intravenous jugular vein catheter and bilateral microinjection cannulae in the NAc core under isoflourane anaesthesia. After recovery, rats were trained to self-administer intravenous METH (0.1mg/kg/infusion) by lever press during 2-hour sessions under a fixed ratio 1 schedule for 20 days. Following extinction of lever press activity,the effect of microinjecting, saline, oxytocin (0.5 pmol, 1.5 pmol, 4.5 pmol) or co-administration of oxytocin (1.5 pmol) and desGly-NH2,d(CH2)5[D-Tyr2,Thr4]OVT (1 nmol, 3 nmol) in the NAcc (500 nl/side) was examined on METH-primed reinstatement (1mg/kg, i.p.). Our results showed that oxytocin administration in the NAc core decreased METH-induced reinstatement in a dose dependent manner, and that desGly-NH2,d(CH2)5[D-Tyr2,Thr4]OVT reversed lever press activity to those exhibited by the control METH day. These findings highlight that oxytocin modulation of the NAc core is an important mediator of relapse to METH abuse. (249 words).

POS-WED-086

PHYSICAL EXERCISE IMPROVES AGE-ASSOCIATED COGNITIVE DEFICITS

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Voluntary physical exercise has been shown to positively effect hippocampal neurogenesis in both young and old animals. In the present study we demonstrate that 24-month-old mice retain a population of neural precursor cells in the dentate gyrus of the hippocampus that can be activated after physical exercise. We also demonstrate that activating these endogenous latent precursor cells correlates to increased neurogenesis and improved cognitive ability.

Using an active place avoidance (APA) paradigm, we assessed spatial memory and learning in young (10-week-old) and old (24-month-old) female mice. Unlike young animals, which were able to learn the location of the shock zone and avoid it, old animals were unable to do so and received a significantly higher number of shocks on each day of testing ($p<0.001$). After initial testing, animals were housed either with or without access to running wheels for 35 days. Following exercise, 24-month-old mice were retested in the APA paradigm and were found to have improved cognitive function, as indicated by receiving fewer shocks, compared to age-matched sedentary controls. The performance of the exercised cohort was comparable to young animals.

Our results demonstrate that physical exercise can ameliorate cognitive decline in mice of old age. By further defining the mechanisms involved in exercise-mediated cognitive improvements it may be possible to develop effective strategies to combat age-associated cognitive deficits. (219 words)

POS-WED-087

FEASIBILITY STUDY TO STUDY CORRELATES OF PRE- AND POSTNATAL BONDING IN (EXPECTING) MOTHERS IN A PUBLIC HOSPITAL SETTING

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3 School of Medical Sciences, The University of Adelaide, Adelaide, Australia.
Objective: Oxytocin (OT) plays a role in mother-infant bonding and is correlated to maternal behaviour in animals. The study assessed the feasibility of measuring pre- and post-natal bonding behaviour and OT levels in women prior to and just after birth in a public hospital setting.

Method: Participants were recruited in the Women's and Children's Hospital Adelaide from a general antenatal midwifery clinic over a three-month period. Saliva OT samples were collected in the third trimester and on postnatal day (PND) 3 before and after an interaction task and analysed using ELISA. Mother-infant bonding was measured using the Maternal Antenatal and Postnatal Attachment Scales and behavioural observations during a mother-child interaction on PND3. Sample collection and assessments took place at the hospital. The interaction task was video taped and coded by a blind coder using the Postnatal Interaction Observation Scale (PIOS)**.**

Results and Conclusion: The number of eligible women was much lower than expected. During the recruitment period, 7 (54%) women agreed to participate; only 3 completed all test days. Although data are not representative of the larger population, the study has demonstrated the suitability of the two assessments tools and of the interaction task in a clinical setting. The PIOS was suitable for scoring maternal behaviour, but not for the interaction due to the young age of the infants. Oxytocin samples were collected correctly and the reported range was similar to the literature. Large individual differences were shown postnatally at baseline; however, interestingly levels were comparable after the interaction task. (word count 250).

POS-WED-088

COGNITION CAN BE IMPROVED WITH BARDOXOLONE METHYL TREATMENT IN MICE FED A HIGH FAT DIET

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Recently, studies have shown that pentacyclic triterpenes have anti-inflammatory effects in the brains of obese mice; a disease where inflammation contributes to energy balance deregulation, and cognitive impairment. Bardoxolone Methyl (BM) is a compound synthetically modified from the pentacyclic triterpene, oleanolic acid. We found that BM treatment in 12 week old C57B1/6 male mice fed a high fat diet (HFD) for 21 weeks significantly prevented body weight gain, and reduced visceral fat, liver weight and liver lipid content compared with untreated mice ($p<0.05$). Furthermore, analysis of BM treated mouse liver tissue revealed significantly reduced levels of PTP1B, an inflammatory mediator, compared to the HFD control group ($p<0.05$). The preference index (PI) determined from a novel object recognition test was significantly higher in BM treated mice by 26.94% compared to untreated HFD fed mice ($p<0.05$). Signalling molecules involved in cognition are currently being analysed using western blotting. Our results indicate that BM induces negative energy balance, reduces liver inflammation and improves recognition memory. (162 words)

POS-WED-089

EFFECTS OF POSITIVE ALLOSTERIC MODULATORS OF M4 MUSCARINIC RECEPTOR ON THE PHARMACOLOGICAL DISRUPTION OF PREPULSE INHIBITION IN MICE.

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Schizophrenia is a devastating disorder that affects about 1% of the population. Current treatments are only efficient in treating a portion of the symptoms and are often associated with a range of side effects. Recently, the M4 muscarinic acetylcholine receptor (mAChR) has been described as a novel treatment target for schizophrenia, with two structurally similar positive allosteric modulators (PAMs) of this receptor, LY2033298 and ML253, offering the potential of a more selective, targeted mode of action[1,2]. To investigate the antipsychotic potential of M4 mAChR PAMs, we used an established model of aspects of schizophrenia, disruption of prepulse inhibition (PPI) in mice induced by MK801, a non-competitive NMDA receptor antagonist. We observed that ML253 at both 3 and 30 mg/kg, when treated in combination with an acetylcholinesterase inhibitor, donepezil, at 3 mg/kg, was able to reverse the disruption of PPI induced by MK801 at prepulse 18 (P=0.025 and P=0.001, respectively). In addition, it has been shown that antipsychotic-like actions can be achieved by targeting M4 mAChRs that are co-expressed with D1 dopamine receptors (DRs) in the striatum[3]. Therefore, we investigated the effect of LY2033298 in the disruption of PPI induced by R-6-Br-APB, a selective D1 DR agonist. We observed that LY2033298 10 mg/kg was able to reverse R-6-Br-APB-induced disruption of PPI (overall reversal of PPI, P=0.006). These results suggest that selective positive allosteric modulation at the M4 mAChR is effective in reversing deficits of sensorimotor gating induced by R-6-Br-APB, but combined donepezil treatment is required to reverse deficits induced by MK801. (250 words)

1. Leach et al. (2010) *Neuropsychopharm* 35, 855–869

2. Le et al. (2012) *Bioorg Med Chem Lett* 23(1):346-50
3. Dencker et al. (2011) *J Neurosci* 31(16):5905–5908

POS-WED-090

ACTIVITY IN THE INFRALIMBIC CORTEX AND NUCLEUS ACCUMBENS SHELL IS ASSOCIATED WITH METHAMPHETAMINE PLACE PREFERENCE.

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Methamphetamine (METH) is a highly addictive psychostimulant for which there are currently no therapeutic treatments. A potential therapeutic target is the adenosine 2A (A_{2A}) receptor, as this receptor modulates the rewarding properties of METH in a place preference paradigm. Our current studies sought to determine the neural locus at which A_{2A} modulates METH reward. A2A *loxP/loxP* and wild type littermates were tested in a place preference paradigm, being conditioned for four consecutive days with either 2mg/kg METH or saline i.p. (n = 8 / treatment). At test, the preference for the METH- or saline-paired context was assessed, and brains perfused following test. c-Fos immunohistochemistry was conducted and manual cell counts were performed throughout the brain. The conditioning protocol produced a significant preference for the drug-paired side in mice conditioned with METH ($p < .01$), but no preference in mice conditioned with saline ($p = .3$). We also observed greater Fos expression in METH-conditioned animals in the infralimbic cortex and nucleus accumbens shell (both p's <.05). These data suggest the expression of METH place preference may be modulated the infralimbic cortex and nucleus accumbens shell. These regions overlap with the known distribution of A_{2A} receptors. Future studies will seek to confirm the involvement of A_{2A} in METH reward using double labelling for Fos and A_{2A} following a place preference test, and conditional deletion of A_{2A} in specific target regions. (227 words)

POS-WED-092

BEHAVIOURAL AND NEURAL CORRELATES OF EXTENDED NICOTINE SELF-ADMINISTRATION IN RATS

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With extended drug use, drug-seeking can transition from a voluntary, goal-directed action towards a habitual and eventually compulsive behavior. The present studies addressed the consequences of extended nicotine self-administration through examining sensitivity to outcome value and motivation for the drug reward following either brief (10 days) or extended (>40 days) intravenous nicotine self-administration. A single pre-session infusion of nicotine increased the latency to the first response following brief, but not extended nicotine self-administration, suggesting a decreased sensitivity to the immediate value of the reward following extended training. Halving the infusion dose had opposite effects at the two time points – a reduction in responding was evident following brief training, whereas an increase in responding occurred following extended training. This was followed up with full dose-responses curves, revealing a leftward shift of the dose-response curve with extended access to nicotine, indicating sensitization to the rewarding effects of nicotine across training. Motivation for nicotine was tested at these two time points using a progressive ratio schedule of reinforcement - following extended self-administration rats worked twice as hard to receive infusions of nicotine. These changes in behavior were paralleled by a shift in nicotine-induced neuronal activation from the ventral to the dorsal striatum, a finding that is consistent with the possibility that extended training results in changes to the site of response control within the striatum. Together these results provide further evidence of changes in the nature of responding for nicotine with extended self-administration that is paralleled by changes in neuronal activation. (248 words).

POS-WED-093

THE ROLE OF MGLU5 IN FEAR EXTINCTION IN THE DEVELOPING RAT

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The metabotropic glutamate receptor 5 (mGlu5) has been implicated as having a role in fear extinction. mGlu5 are developmentally regulated, therefore we investigated the contribution of mGlu5 in fear extinction in developing rats, by pharmacologically modulating mGlu5 signalling using negative (MTEP) and positive (CDPPB) allosteric modulators of mGlu5. Rats aged postnatal day (P) 16 (juvenile) and P23 (pre-adolescent) were trained to fear a white-noise conditioned stimulus (CS) by pairing it with a foot-shock. To decrease or increase mGlu5 signalling, MTEP (2mg/kg), CDPPB (3mg/kg) or vehicle were injected systemically prior to extinction on the following day. When rats were re-tested for CS elicited fear, both P17 and P24 rats previously injected with MTEP froze significantly more than those injected with vehicle ($P<0.05$), suggesting that MTEP impaired extinction (Experiment 1). Conversely, pre-extinction injection of CDPPB led to significantly less freezing compared to vehicle on test day regardless of age ($P<0.05$), suggesting that CDPPB facilitated extinction in both age groups (Experiment 2). Taken together it appears that in developing rats glutamatergic signalling via mGlu5 may have an important role in fear extinction, and enhancement of mGlu5 signalling may offer an alternative therapeutic target for treating anxiety disorders in these age groups. (198 words)

POS-WED-094

VITAMIN B12 DEFICIENCY *PER SE* IN EARLY LIFE LEADS TO ANXIETY IN MICE DURING ADULTHOOD

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Imbalance in the nutritional environment during the early phase of life acts as a stressor and can translate into mental health problems in adulthood. Considering that (i) vitamin B12 deficiency is largely prevalent in pregnant and lactating women in developing countries like India and (ii) vitamin B12 deficiency interferes with normal brain development and cognition, we have created a mouse model of the same in order to study the alteration in behavior and the underlying molecular, including epigenetic, changes. Female, weanling C57BL/6 mice received *ad libitum* a control diet (American Institute of Nutrition-76A) or the same with restriction of vitamin B12 for 12 weeks. After confirming the B12 deficiency status, we subjected these mice to a battery of behavioral tests – open field activity, light and dark exploration, elevated plus maze and social interaction, to assess if there is any behavioural alteration induced by vitamin B12 deficiency. Golgi-Cox staining was done to assess changes in neuronal morphology, if any. The behaviour data indeed reflects development of anxiety in vitamin B12 restricted mice. The Golgi-Cox staining shows that neuronal morphology and the connections between neurons in prefrontal cortex are affected in B12 deficient mice. Our preliminary results on underlying molecular mechanisms show that hyperanxiety in vitamin B12 deficient mice is due to changes in histone acetylation/ deacetylation and methylation/ demethylation in prefrontal cortex and hippocampus. This study suggests that deficiency of vitamin B12 *per se* during early phase of life leads to the development of anxiety behavior in C57BL/6 mice in adulthood.

POS-WED-095

NOVEL POSITIVE ALLOSTERIC MODULATORS OF THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR DEMONSTRATE SAFE, EFFECTIVE COGNITIVE ENHANCEMENT IN MICE AND RATS.

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Positive allosteric modulation (PAM) of the α7 neuronal subtype of the nicotinic acetylcholine receptor (nAChR) represents a promising therapeutic strategy for cognitive enhancement in disorders including Alzheimer's disease and schizophrenia. Compared with α7 nAChR full or partial agonists, PAMs do not inhibit nAChRs by desensitization instead their effect is unequivocally potentiating. PAMs also amplify nAChR transmission without affecting intrinsic spatio-temporal signalling patterns, and are generally more subtype-selective.

Three novel α7 PAMs, BNC1881, BNC2591 and BNC375, were developed using fluorescence screening of Bionomics' focussed library of novel compounds and subsequent medicinal chemistry campaign. These compounds were characterised in a Ca^{2+} flux assay, electrophysiology, and in behavioural models of cognition: the mouse T-maze Continuous Alternation Task (T-CAT) and the rat novel object recognition (NOR) model of non-spatial memory.

At 3μM all three compounds potentiated an EC_{20} of nicotine-evoked calcium flux in a fluorescence-based assay or acetylcholine-induced currents in automated planar and conventional patch-clamp assays in rat GH4C1 α7 nAChR-expressing cells.

When tested in vivo, all compounds were tolerated well up to the highest dose tested (30 mg/kg) and showed a significant dose-dependent reversal of scopolamine-induced memory impairment. BNC2591 and BNC375 also demonstrated *in vivo* efficacy when administered orally.

In summary, the novel α7 nAChR PAMs, BNC2591, BNC1881, and BNC375, demonstrated safe, effective cognitive enhancement in mice and rats. Based on results of in vitro and in vivo characterisation as well as pharmacokinetic properties α7 nAChR, BNC375 was selected as Bionomics' drug candidate for further development.

(Word count: 246)

POS-WED-096

IMPACT OF ADULT VITAMIN D DEFICIENCY ON HIPPOCAMPAL NEUROGENSIS IN BALB/C MICE

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Recent animal experiments have demonstrated that adult vitamin D (AVD) deficiency is associated with altered behavior and neurochemistry. Epidemiological evidence links low vitamin D with cognitive decline, therefore there is a need to explore the impact of this common exposure on more specific neurobiological processes. This study examines the impact of AVD deficiency on adult hippocampal neurogenesis in mice.

Adult BALB/c mice were fed a control or vitamin D deficient diet for 10 weeks, and then individually housed with or without running wheels to stimulate hippocampal neurogenesis. The mice were treated with BrdU for 10 days and then left for 5 weeks to assess hippocampal neurogenesis. Prior to collection of brain tissue the mice were tested for locomotion on open field.

Wheel running had a beneficial effect on adult hippocampal neurogenesis in all mice, however in preliminary results, this effect was blunted with AVD deficiency leading to reductions in hippocampal neurogenesis compared to controls ($p<0.05$). There were differential behavioural effects of AVD deficiency on male and female mice. AVD-deficient females did not show enhanced wheel running, which may explain the reduced neurogenesis. By contrast, AVD-deficient males, which showed increased wheel running, were less active than control mice in a novel open field, in addition to reduced hippocampal neurogenesis.

We report, for the first time, that AVD deficiency impacts on hippocampal neurogenesis and that this may be sex-specific. Mindful that one in three Australians has insufficient vitamin D concentrations, it is important to uncover the neurobiological consequences of vitamin D deficiency. (250 words)

POS-WED-097

INSENSITIVITY OF EEG GAMMA PEAKS TO EXTERNAL STIMULATION.

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Approximately 20% of individuals express atypical peaks in power of electroencephalographic (EEG) activity in the gamma range (25-100 Hz). We investigated whether these gamma peaks participate in sensory and/or cognitive processes.

128-channel EEG was recorded from subjects in whom gamma peaks had previously been observed whilst they undertook sensory and cognitive tasks.

Sensory: visual, auditory, and tactile steady-state stimulation was used to stimulate cortical sensory oscillations (steady-state response, SSR) at the frequency of the individual's gamma peak and at surrounding frequencies.

Cognitive: a visual, auditory, and tactile oddball paradigm (detection of a rare 'target' stimulus) was used to investigate the interaction of the individual's gamma peak with cognitive gamma responses induced/evoked by the task. We also added a difficulty manipulation to determine whether the gamma peaks participated in enhanced attention.

SSRs at the gamma peak frequency were not significantly different from stimulation at other frequencies. There were no cognitive effects at the gamma peak frequency during the oddball task.

In conclusion, we found no evidence that gamma peaks are involved in either sensory or cognitive function. (176 words).

POS-WED-098

BEHAVIOURAL AND NEUROIMMUNOLOGICAL RESPONSES TO EARLY LIFE STRESS (ELS) AND SUBSEQUENT CHRONIC MILD STRESS (CMS) IN C57BL/6 MICE.

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Background: Preliminary results suggest that ELS alone may not be enough to elicit a behavioural change in adult C57BL/6 mice. Therefore we hypothesise, that C57BL/6 mice undergoing maternal separation (MS) would be predisposed to behavioural and immune function changes following exposure to a 'second-hit' of CMS in adulthood.

Methods: We exposed C57BL/6 mice to either a) no stressors, b) MS, c) CMS d) MS+CMS. We measured behavioural changes, corticosterone and NGF levels in hippocampal formation. We measured various immune responses using flow cytometry.

Results: CMS mice alone displayed more depressive-like behaviour than MS ($p=0.0071$) or MS+CMS groups ($p=0.0381$) and less anxiety-like behaviour than MS ($p=0.0018$) and the control group ($p=0.0118$). NGF levels were decreased only immediately after exposure to MS ($p=0.0027$). T-cell numbers were significantly decreased only in MS mice compared with all other groups (MS $p=0.0026$; CMS $p=0.0418$; MS+CMS $p=0.0178$). Single stressors had T-cells characterised by an activated/memory phenotype ($CD62L^{lo}$, CD25+ and $CD44^{hi}$) when compared with the control group ($p<0.05$) Intracellular TNF was not increased in T-cells ($p<0.05$) or in monocytes ($p<0.01$) of all stress groups compared to controls.

Conclusions: Our results indicate that MS doesn't predispose C57BL/6 mice to behavioural changes following CMS in adulthood. However, exposure to CMS alone induces depressive-like but not anxiety-like behaviour. Single stressors appear to contribute to reducing peripheral T cells by altering T cell homing, survival and homeostatic proliferation. Results from the MS+CMS group suggest that a combination of stress events might counteract any neuroimmunological changes observed after a single stress event. (250 words)

POS-WED-099

NEUROPROTECTIVE EFFECTS OF ESTRADIOL ON SPATIAL MEMORY ARE REVERSED IN BDNF HETEROZYGOUS KNOCKOUT MICE

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Both estradiol and Brain-Derived Neurotrophic Factor (BDNF) have neuroprotective properties, particularly with respect to cognition. We sought to determine the effects of pre-pubescent ovariectomy (OVX) and estradiol replacement on spatial memory (assessed in the Y-maze) and hippocampal BDNF protein expression (measured by Western blot) in young adult wild-type mice (WT) and BDNF heterozygous mice (+/-).

OVX significantly impaired spatial memory in WT ($p=0.015$) which was restored by estradiol replacement ($p=0.035$). Intact $BDNF^{+/-}$ mice showed a significant deficit in spatial memory compared to intact WT ($p=0.015$). However and unexpectedly, OVX improved this spatial memory deficit as $BDNF^{+/-}$ OVX mice performed significantly better than WT OVX ($p=0.04$). Furthermore, estradiol replacement reversed this positive effect of OVX in $BDNF^{+/-}$ mice. OVX significantly reduced BDNF expression in the dorsal hippocampus (DHP) of WT ($p=0.02$), while estradiol replacement significantly restored levels to those of intact controls ($p=0.02$). As expected, BDNF expression was significantly reduced in the DHP of $BDNF^{+/-}$ ($p=0.03$), however OVX and OVX + estradiol had no further impact on BDNF protein expression in the $BDNF^{+/-}$ mice.

These data confirm a neuroprotective role for estradiol in spatial memory and suggest that BDNF may mediate this effect in WT mice. Interestingly, this neuroprotective role of estradiol is reversed in $BDNF^{+/-}$ mice. This altered response to estradiol in $BDNF^{+/-}$ mice may be of relevance to human schizophrenia and major depression patients carrying polymorphisms in the BDNF gene. (231 words)

POS-WED-100

THE EFFECT OF THE ANTI-PSYCHOTIC DRUG QUETIAPINE AND ITS METABOLITE NORQUETIAPINE ON ACUTE INFLAMMATION

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Objective: Quetiapine is an antipsychotic drug which has also been used in bipolar disorder, major depressive disorder and general anxiety disorder. It has recently been suggested that it may also have an anti-inflammatory effect which could be important for the inflammatory aspects of various psychiatric diseases or comorbid inflammatory conditions such as arthritis. **Methods:** Male C57BL/6 mice were given either quetiapine (10 mg/kg), its main active metabolite norquetiapine (10 mg/kg) or saline as a vehicle control once a day for 14 days. On the 14th day, this dose was followed by a single dose of either LPS (1 mg/kg) or saline. 24 hrs following LPS or saline, mice were tested in the Y-maze and immediately following behavioural testing, mice were culled. Serum, prefrontal cortex and hippocampus protein analysis of cytokine levels was conducted. **Results:** Y-maze results show that LPS led to impaired cognition in the Y-maze which was not prevented by pre-treatment with either quetiapine or norquetiapine. LPS led to changes in serum levels of various cytokines in saline and quetiapine pre-treated mice, however no changes in IL-6 and MCP-1 were seen in norquetiapine pre-treated mice. Quetiapine pre-treatment appeared to prevent increases in hippocampal cytokine levels 24 hr post LPS. **Conclusions:** The results suggest that norquetiapine may prevent some of the changes in serum cytokine levels seen 24 hr following LPS, and quetiapine some of the cytokine changes seen in the hippocampus, however neither drug affects the detrimental effect of LPS on cognition. (243 words)

POS-WED-101

EXAMINING THE OLIGOMERIZATION, BINDING AND NEUROTOXIC PROPERTIES OF Aβ PEPTIDES ASSOCIATED WITH ALZHEIMER'S DISEASE

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BACKGROUND: The presence of amyloid β (Aβ) plaques in brains is a key requirement for describing the pathogenesis of Alzheimer's disease (AD). While the level of soluble oligomeric Aβ species in AD brains correlates best with the development of AD symptoms, the neurotoxic Aβ species remains elusive. We propose that Aβ binding to neurons is crucial to mediate neuronal dysfunction and toxicity. This study aims to identify the oligomeric Aβ species bound to neurons over time to correlate with neurotoxicity. *METHODS:* Mouse primary cortical neurons were cultured 6 days *in vitro* before treatment with monomerised $Aβ_{40}$ or $Aβ_{42}$ peptides over time. Cell viability and Aβ binding profiles were determined by CCK8 assay and western blotting respectively. The Photo-induced cross-linking of unmodified peptide (PICUP) technique was used to prepare oligomeric Aβ species. Monomer to tetrameric Aβ oligomers were individually purified and their toxicity and binding to neurons assessed after 96 hr treatment. *RESULTS:* We observed that the amount of total Aβ bound to neurons correlated with the level of toxicity. We found that only trimeric and tetrameric Aβ binding to neurons showed a significant correlation with Aβ-induced neurotoxicity, while monomer and dimer binding did not correlate with toxicity. This was further supported with purified PICUP-induced Aβ trimers and tetramers inducing significant toxicity, while monomer and dimeric species were not toxic. *CONCLUSION:* This study showed significant correlation between the binding of Aβ trimer and tetrameric oligomers and Aβ-induced neurotoxicity. We propose they are the key toxic species causing AD pathogenesis. (248 words)

POS-WED-102

MATERNAL SEPARATION STRESS, A MODEL OF EARLY LIFE STRESS: UNDERSTANDING THE NEURO-IMMUNE INTERACTION EFFECT ON ADULT PSYCHIATRIC ILLNESSES

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Background: Early life stress (ELS) events such as childhood maltreatment have been shown to increase inflammation in adulthood. Maternal separation (MS) stress, an animal model of ELS has been implicated in behavioural and neurobiological alterations in adulthood. However, the neuro-immune interaction effects of MS on adult behaviour and biology are unclear. We hypothesize that neuro-immune interactions post MS are dependent on the type of MS and may have a role in long-term effects of MS in adulthood.

Method: We used the C57BL/6 mouse strain and two different types of separation: a) MS with pups separated as a group (MS-G) and b) MS with pups separated individually (MS-I). MS was conducted for 3 hours every day between postnatal days 1-14. We then assessed the effect of MS on adult (8-10 weeks) behaviour, neurobiology and immune response.

Results: MS-I mice showed a significant increase in locomotor activity, decreased anxiety-like behaviour and impaired learning compared to the control and MS-G mice. Analysis of T cell numbers and early activation status showed no difference between the groups however cytometric bead array analysis showed an increase in IL-10 in the MS-I group compared to controls and MS-G.

Conclusion: Our results show that MS-I might have greater effects on adult behaviour and biology compared to MS-G. The increased IL10 in MS-I mice could be a compensatory anti-inflammatory mechanism directly proportional to the level of inflammation after MS. We infer that a second stress treatment in adolescence or adulthood may be needed for better neuro-immune interaction response. (250 words)

POS-WED-103

THE ROLES OF THE LATERAL HABENULA AND BASOLATERAL AMYGDALA IN PUNISHMENT

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Volitional behaviour that causes an aversive outcome will be suppressed, i.e. punished. The neural bases of punishment are poorly understood. Two regions implicated in punishment are the lateral habenula (LHb), which inhibits midbrain dopamine neurons in response to aversive stimuli, and the basolateral amygdala (BLA). The current experiments studied the roles of the LHb and BLA in punishment. During Stage I, rats were trained to respond on two individually-presented levers that caused the delivery of sucrose pellets. During Stage II, one of these levers caused delivery of a footshock as well as sucrose pellets (punished lever) while the other lever continued to only deliver sucrose pellets (unpunished lever). Rats rapidly reduced responding on the punished lever over the course of Stage II. Infusions of the AMPA antagonist NQBX into the LHb had no effect on the acquisition or expression of this punishment, regardless of whether the levers were presented individually or together in discrete choice test. Infusions of baclofen/muscimol into the BLA attenuated the acquisition and expression of punishment when the levers were presented individually but not during a choice test. Similar results were observed after inhibition of BLA glutamatergic neurons via AAV expression of the hM4Di DREADD. These findings suggest that BLA, but not LHb, is important for the primary punishing effects of shock.

(Word count: 215)

POS-WED-104

EFFECT OF APOCYNIN ON DISRUPTIONS IN BEHAVIOUR AND BRAIN PARVALBUMIN IMMUNOREACTIVITY IN THE SUBCHRONIC PHENCYCLIDINE RAT MODEL OF SCHIZOPHRENIA.

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A sub-chronic administration of phencyclidine to the rat brings about enduring pathophysiological and cognitive changes that resemble some features of schizophrenia. The present study aimed to determine if concurrent administration of apocynin, an inhibitor of NADPH oxidase activity, could attenuate the effect of phencyclidine on behaviour and parvalbumin-containing neurons in the prefrontal cortex and hippocampus. Rats were administered phencyclidine at a dose of 5 mg/kg i.p. bi-daily for 1 week, or vehicle. Half of the phencyclidine group was concurrently treated with apocynin (5 mg/kg via the drinking water, 7 days before, 7 days during and 7 days after phencyclidine administration). Novel object recognition memory, locomotor activity, and subsequent brain analysis were assessed 1 week post-phencyclidine treatment. Deficits in phencyclidine behaviour did not appear to be affected by concurrent apocynin treatment. However post-phencyclidine analysis of brains demonstrated a reduction in expression of parvalbumin-immunoreactive neurons that were in part rescued by concurrent apocynin treatment in regions of the prefrontal cortex (infralimbic cortex $p<0.05$), and hippocampus ($p<0.05$).
These results show the importance of the sub-chronic phencyclidine rat in modelling the behavioural deficiencies and brain pathology observed in schizophrenia, and suggest that concurrent apocynin is neuroprotective in this model.

POS-WED-105

CLOZAPINE REVERSES THE ELECTROPHYSIOLOGICAL ABNORMALITIES AND ASSOCIATED DISRUPTION OF PREPULSE INHIBITION CAUSED BY NMDA RECEPTOR ANTAGONISTS

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Objective: Recent heuristic models of schizophrenia propose that dysfunction in the generation of synchronous gamma (30-80Hz) oscillatory activity may be related to the pathophysiology of the disorder. This study assessed the effects of antipsychotics on gamma oscillatory abnormalities and PPI deficits in the NMDAr antagonist animal model of schizophrenia

Methods: EEG electrodes were implanted into adult male Wistar rats (n=6). Rats were administered one of five antipsychotics: haloperidol (0.25mg/kg), clozapine (5mg/kg), LY379268 (3mg/kg), NFPS (1mg/kg), D-serine (1800mg/kg) or vehicle, and 25 minutes later were administered MK-801(0.16mg/kg) or vehicle. Measures of ongoing gamma power, prepulse-evoked gamma power and %PPI were assessed.

Key findings: MK-801 significantly elevated ongoing gamma power and reduced prepulse-evoked gamma power, both of which were temporally related to drug-induced disruptions in %PPI. Haloperidol, clozapine and LY379268 all significantly reduced the MK-801 induced elevation in ongoing gamma power. However, only clozapine significantly ameliorated the MK-801 induced evoked gamma power deficit. In addition, clozapine was the only antipsychotic that reversed the PPI deficit, suggesting that these effects are related. The NMDA receptor enhancing agents, D-serine and NFPS, had no effects.

Conclusion: The ability of neural networks to upregulate gamma oscillatory activity in response to the processing of stimuli may be more relevant to sensorimotor gating ability than the background level of gamma oscillations. Moreover, considering deficits in evoked gamma power are associated with treatment resistant symptoms in schizophrenia, clozapine's unique ability to ameliorate deficits to evoked gamma power here may relate to its superior clinical efficacy in treatment resistant schizophrenia.

(250 words)

POS-WED-106

RELAXIN-3/RXFP3 SYSTEM REGULATES STRESS-INDUCED REINSTATEMENT OF ALCOHOL-SEEKING IN BED NUCLEUS OF STRIA TERMINALIS IN RATS

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Alcoholism is a chronic relapsing disorder, accounting for 10% of disability-adjusted life years lost in industrialized countries. Stress is a key cofactor in relapse, and relaxin-3/RXFP3 signalling is implicated in the regulation of stress responses. Central antagonism of RXFP3 can reduce alcohol consumption and seeking and we therefore tested whether RXFP3 antagonism in stress-related brain areas, such as the bed nucleus of stria terminalis (BNST), modulated alcohol use and stress-induced alcohol-seeking. Rats were trained to self-administer ethanol (10%) on a FR3 ratio and then underwent stereotaxic surgery to position intracerebral guide cannulae into the lateral ventricle or immediately above the BNST. After recovery and re-stabilisation, rats received bilateral injections of vehicle or the RXFP3 antagonist, R3(B1-22)R (1 µg/0.5 µl) into the BNST and were tested for ethanol self-administration. Reinstatement was performed after 10-14 days of extinction using pre-treatment with the pharmacological stressor, yohimbine (1 mg/kg, i.p.). Intracerebroventricular R3(B1-22)R (10 µg/5 µl) prevented stress-induced reinstatement of alcohol-seeking ($F_{(2,18)}$ 15.00, $p = 0.0001$), but had no effect on stress-induced reinstatement of sucrose-seeking, indicating a specific effect.
Microinjection of R3(B1-22)R in the BNST (1 µg/0.5 µl bilaterally) decreased self-administration of alcohol ($F_{(2,34)}$ 8.850, $p = 0.0008$) and markedly attenuated stress-induced reinstatement of alcohol-seeking ($F_{(2,22)}$ 16.77, $p< 0.0001$). Studies to elucidate the mechanism underlying these effects are currently underway. These data suggest the relaxin-3/RXFP3 system can modulate both alcohol consumption and stress-induced reinstatement of alcohol-seeking; and highlight the need to better elucidate the neurocircuitry of relapse-like behaviour. (243 words)

POS-WED-107

UTILISING TOUCHSCREEN TECHNOLOGY FOR THE PRECLINICAL MODELING OF COGNITION IN A SCHIZOPHRENIA MOUSE MODEL

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The complex profile of cognitive impairment in schizophrenia, like many other psychiatric disorders, is poorly understood. The role of glutamatergic signalling in cognitive impairment is well recognized. Metabotropic glutamate receptor 5 (mGluR5) has been associated with schizophrenia and mGluR5 knockout (KO) mice have been shown to exhibit behavioural abnormalities regarded as endophenotypes of relevance to schizophrenia. With the recent development of touchscreen operant behavioural testing for rodents we are now able examine multiple discrete forms of learning and memory in a mouse models of schizophrenia. We aimed to assess discrimination learning in mGluR5 KO mice using the touchscreen visual discrimination task. In this task mice selected, via nose-poke, one of two digitally displayed stimuli on a touch sensitive screen. Correct selections were rewarded with a small amount of strawberry milkshake. mGluR5 KO mice demonstrated significant impairments in task acquisition compared to their wild-type littermates. Upon successful acquisition the mice were assessed on a reversal learning task. In this task the stimuli were reversed, so that the previously unrewarded stimulus was rewarded and vice versa. In the initial two days of the reversal learning task the mGluR5 KO mice were significantly more perseverative, indicating an impairment in cognitive flexibility. We are currently extending the cognitive battery using this technology and exploring both molecular and cellular aspects of cognitive deficits. Our results highlight a critical role for mGluR5 in discrimination learning and cognitive flexibility and may inform the future development of treatments for cognitive dysfunction in brain disorders such as schizophrenia. (249 words).

POS-WED-108

DOSE-DEPENDENT MK-801 INDUCED-SENSITISATION IN THE MALE SPRAGUE-DAWLEY RAT

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Objective: The behavioural and neurochemical adaptations associated with drug-sensitisation are thought to be relevant to the pathophysiology of schizophrenia and drug addiction. Agents that manipulate dopamine release or uptake are typically used in these studies. However, understanding the neural mechanisms of sensitisation to MK-801, a potent non-competitive NMDA receptor antagonist, could provide insight into the glutamate pathophysiology of schizophrenia. The main aim of this study was to identify the optimal dose required for robust sensitisation.
Methods: Male Sprague-Dawley rats (n=120) were assigned to one of four MK-801 dose treatment groups: Saline, Low (0.1mg/kg), Med (0.25mg/kg) or High (0.5mg/kg). Daily injections were administered in the test cage for 7 days to induce sensitisation. Following a 5 day withdrawal period, each group was administered either a Low, Med or High MK-801 drug challenge to elicit locomotor sensitisation.
Results: Induction with Med MK-801 significantly enhanced subsequent locomotor responses to challenges with this same dose ($p<0.05$). However, induction with saline also significantly enhanced the locomotor response to Med and High MK-801 challenge ($p<0.05$). Induction with the Low dose had no significant enhancement to challenge with any dose. Induction with the High dose resulted in a median split of ‘sensitisers’ and ‘non-sensitisers’ based on individual change in locomotor activity.
Conclusions: This study has identified a robust MK-801 sensitisation regime using repeated Med MK-801 in a novel environment. Unexpectedly, repeated saline injections were sufficient to induce a sensitised MK-801 response, suggesting cross-sensitisation to the procedure (such as injection or context). Further studies are required to understand this phenomenon. (248 words).

POS-WED-109

THE EFFECT OF ANTI-INFLAMMATORY TREATMENTS ON BEHAVIOUR IN A GFAP-TNF$^{+/+}$ MOUSE MODEL OF NEURO-INFLAMMATION.

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Background: Inflammation is important in the patho-biology of neuropsychiatric conditions. It has been postulated that anti-inflammatory treatments such as exercise and non-steroidal anti-inflammatory agents (NSAIDs) may be effective in the treatment of neuropsychiatric diseases, but little is known about their effect on behaviour.

Methods: GFAP-TNF$^{+/+}$ transgenic mice (a mouse model of neuro-inflammation) and wild-type (WT) C57BL/6 mice (n=10/grp) were divided into exercise treatment (21 days voluntary wheel running), NSAID treatment (2.5 mg/kg indomethacin, i.p. twice daily, 7 days), or control (no treatment) groups. After treatment, mice were compared in locomotion, spatial recognition memory, anxiety-like and depression-like behaviour using a battery of behavioural tests.

Results: In GFAP-TNF$^{+/+}$ mice, both exercise (p=0.009) and indomethacin groups (p=0.027) showed lower baseline loco-motor activity than control mice. In addition, spatial recognition memory was impaired in the indomethacin group, which also exhibited higher levels of anxiety-like behaviour than untreated control mice (p=0.045). GFAP-TNF$^{+/+}$ exercise mice exhibited higher depression-like behaviour than control (p=0.0041) and indomethacin mice (p=0.0001). Similarly, WT indomethacin treated mice showed impaired spatial recognition memory, and exhibited higher anxiety-like behaviour than controls (p=0.035). No differences in locomotion or depression-like behaviour were observed between treatment groups and controls in WT mice.

Conclusions: Treatment with exercise and indomethacin do not improve and may worsen spatial recognition memory, locomotion, anxiety-like and depression-like behaviour in both WT mice and the GFAP-TNF$^{+/+}$ mouse model of neuro-inflammation. The biological mechanisms underlying the relationship between anti-inflammatory treatments, inflammation and behaviour require further exploration. (241 words).

POS-WED-110

AN IN VITRO STUDY OF THE EFFECT OF EDARAVONE ON THE NEUROTOXICITY OF AMYLOID-B

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Amyloid-β oligomer and oxidative stress are the key factors involved in pathogenesis and development of Alziheimers disease. Edaravone is a strong antioxidant widely used clinically to treat stroke, brain injury and other neurological conditions. Previous studies showed that Edaravone has effect on the cellular APP processing, abeta production and BACE1 expression in cultured SY5YAPP695 cells. In this study, We studied the effect of Edaravone on ROS production, apoptosis and inhibitory effect on neurite growth of cultured primary cortical neurons induced by Amyloid-β oligomers. Quantitative PI labeling studies demonstrated that Edaravone can significantly reduce the number of PI labelled neurons after amyloid-β oligomer treatment. ROS assay showed that Edaravone can significantly reduce the ROS product induced by abeta oligomers. Edaravone can also promote the neurite growth and ameliorate the inhibitory effect on the neurite growth induced by the amyloid-β. In conclusion, Edaravone can significantly protect against the inhibitory effect on neurite growth of cultured cortical neurons induced by amyloid-β oligomers, and ameliorates their the neurotoxic and apoptotic effect on the cultured cortical neurons. The results from this study suggest that Edaravone is a potential therapeutic agent for AD.

POS-WED-111

THE ROLE OF CELL ADHESION MOLECULE CHL1 IN VENTRAL MIDBRAIN DOPAMINE DEVELOPMENT

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The ventral midbrain (VM) dopamine (DA) pathways are important for motor and cognitive function. Consequently dysfunction within these networks has been associated with a number of neurological and psychiatric disorders. Abnormal development may underlie these disorders and thereby highlights the significance of understanding the intricate and precise sequence of events that results in their birth and connectivity. While a number of regulators of VM neurogenesis and DA axon guidance are known, the function of adhesion molecules in these contexts remains to be elucidated. Cell adhesion molecule with homology to L1CAM (CHL1), a member of L1 family, has been shown to influence neuronal survival, proliferation and axon guidance elsewhere in the developing CNS. Here we examine CHL1's function in VM DA development. We demonstrate that CHL1 is expressed in the VM during periods of dopaminergic neurogenesis and axon morphogenesis. Given the ability of CHL1 to function in a soluble and bound form, we examined its function in both contexts on VM primary cultures. CHL1 had no effect on VM neurogeneisis or DA differentiation. Bound CHL1 enhanced DA neurite number, branching and length, while soluble CHL1 increased branching, total DA neurite and DA axon length. These changes were selective to DA neurons, as no changes in neurite morphology were observed in other (non-dopaminergic) neurons in culture. These findings infer that cell adhesion molecule Chl1 may play an important role in the establishment of midbrain dopaminergic pathways. Ongoing studies, in CHL1 mutant embryos, are further examining its role in this context.

POS-WED-112

PURIFIED INTERNEURONS WHEN TRANSPLANTED CAN MIGRATE AND INTEGRATE INTO EMBRYONIC AND POSTNATAL CORTICAL CIRCUITS

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Correct positioning of interneurons results from interaction between the intrinsic properties of interneurons responding to extrinsic signals from surrounding excitatory pyramidal neurons. It is unknown when these interactions occur and what impact such influence has on the integrative properties of interneurons into cortical circuits. We have employed transplantation strategies to address this question. Migrating interneurons were obtained by fluorescent-activated cell sorting (FACS) of embryonic day (E) 15.5 GAD67-knock-in-GFP somatosensory cortex and injected *in utero* into the lateral ventricle of wild-type E15.5 host or into the cortical wall of postnatal day 4 host at the following coordinates from bregma: 0.7mm A, 2.0mm L, 1.2mm D. At set time-points post-transplantation, brain tissue was fixed and processed for immunohistochemistry. In comparison to cells derived from the medial ganglionic eminence, FACS-sorted migrating GFP-positive interneurons were limited in distribution and remained localized to regions within the cortical wall. The interneurons exhibited the morphology of a migrating cell with a prominent cell process extending from the soma, however, the restriction of location was observed in the anteroposterior and mediolateral axes of the cortex. By postnatal day 21, interneurons arborize and expressed subtype-specific markers. Our comparison of integrative capabilities of interneurons along the development migratory route sheds new light on the instructive mechanisms directing placement into cortical layers.

POS-WED-113

REDUCED BMP RECEPTOR IA IN OLIGODENDROCYTE PROGENITOR CELLS ALTERS DIFFERENTIATION AND MYELINATION IN VITRO

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Oligodendrocyte cell death occurs in demyelinating diseases and leads to axons losing their myelin sheaths, which results in disruption to axon conduction. Enhancement of oligodendrocyte regeneration by endogenous progenitor cells (OPCs) is a promising strategy for repair. We have shown that inhibition of Bone Morphogenic Protein (BMP) signalling increases myelin repair in a mouse models of demyelination. BMP signals through two type I receptors (BMPRIa and BMPRIb) and one type II receptor (BMPRII). With the knowledge that BMPRIa is highly expressed in OPCs, we hypothesised that BMPRIa may play an important role in OPC proliferation and differentiation. To test this, we created a new transgenic mouse line with inducible knockout of BMPRIa in OPCs. We used primary cultures of OPCs from these mice to assess the effects of BMPRIa cKO on OPC proliferation and differentiation in vitro and used OPCs co-cultured with dorsal root ganglion neurons to assess the effects of BMPRIa cKO on myelination. We confirmed successful tamoxifen-induced recombination in cultured OPCs. We found that a reduced level of BMPRIa in OPCs does not alter OPC proliferation; however, reduced BMPRIa does alter cell morphology during OPC differentiation. In addition, co-culture experiments suggest that BMPRIa cKO enhances myelination. Future in vivo studies will help inform as to whether BMPRIa in OPCs plays a role in myelin pathology and if disruption to BMP signalling by BMPRIa cKO increases myelin repair, and therefore, constitutes a potential therapeutic target. (236 words)

POS-WED-114

DIFFERENTIATION AND PROLIFERATION IN MOUSE SYMPATHOADRENAL CELLS.

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Adrenal medullary chromaffin cells and peripheral sympathetic neurons appear to originate from a common sympathoadrenal progenitor cell. The time course and signalling mechanisms underlying this segregation during embryonic development are not fully understood. The present study investigates the expression patterns of catecholaminergic synthetic enzymes as well as cell cycle dynamics in the embryonic murine adrenal medulla and the neighboring suprarenal ganglion by immunohistochemical analysis. Transverse sections of adrenal region in E12.5-16.5 C57/bl6 mice were stained with antibodies against tyrosine hydroxylase (TH), phenylethanolamine N-methyltransferase (PNMT), cocaine and amphetamine regulated transcript (CART) and Ki67. Dual-labelling with S-phase markers, bromodeoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) allowed cell cycle analysis. The noradrenergic marker, TH was detected in cells in both the adrenal and sympathetic, with much stronger immunoreactivity for TH in the adrenal. In the suprarenal ganglion, CART was strongly expressed in most neuroblasts whereas it was detected in only a handful of adrenal chromaffin cells. This divergence of phenotype appeared as early as E12.5. The adrenergic marker, PNMT was only expressed in a subpopulation of chromaffin cells from E14.5. Overall, few chromaffin cells were in the cell cycle at E12.5 but the proportion increased on subsequent days until, by E14.5, 40% of the cells were cycling. In contrast, most neuroblasts in the suprarenal ganglion were cycling at E12.5 but then progressively withdrew from the cell cycle. On E14.5, the cell cycle length of noradrenergic chromaffin cells in the E14.5 mice was 30.4 hours versus 11.8 hours in the neuroblasts of the suprarenal ganglion.

(249 words).

POS-WED-115

NOVEL EXPRESSION OF *MICRORNA (MIR)-196A1* AND *MIR- 196A2* IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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The *miR-196* family consists of three miRNAs (*miR-196b*, *miR-196a1*, *and miR-196a2*) which are embedded within the *Hox* clusters. Like their neighbouring *Hox* genes, these miRNAs would be predicted to display co-linear expression reflecting their genomic location within the *Hox* clusters. Using eGFP reporter knock-ins at the *196a1* and *196a2* loci, we have identified novel expression from these loci in the mouse cerebellum, well outside the posterior expression domain normally observed for the neighbouring genes of *Hox* paralogue groups 9 & 10. Both *miR-196a1* and *-196a2* display a stereotyped pattern of expression within the cerebellum, which is grossly maintained from early postnatal periods, when cerebellar circuitry is established, through to adult stages. We are currently investigating the possible role of these miRNAs in establishment or maintenance of the sensorimotor circuitry.

POS-WED-116

VIAMIN D PROMOTES DOPAMINERGIC CELLS DIFFERENTIATION OF VDR-TRANSFECTED HUMAN NEUROBLASTOMA LINE SH-SY5Y

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Purpose: Vitamin D is a neuroactive steroid and acts through the vitamin D receptor (VDR). The VDR emerges in rat mesencephalon at embryonic day 12, the peak period of dopaminergic cell birth. Our evidence reveals that prenatal vitamin D deficiency alters dopaminergic development in rat. However, there is lack of direct evidence showing that vitamin D modulates dopaminergic cells differentiation. The aim of the current study is to investigate this in vitro using a VDR-transfected human SH-SY5Y line.
Method: Rat VDR in pTarget vector was stable transfected into SH-SY5Y. The cells were treated with retinoid acid for 7 days and vitamin D at concentrations of 0.1,1,10,20 and 100nM for another 7 days. The expression of tyrosine hydroxylase (TH) and β-tubulin III were assessed with western blot and immunofluorescence. Cell proliferation was examined using EdU incorporation.
Result: Vitamin D significantly increased TH expression in VDR-transfected SH-SY5Y and this effect was dose-dependent ($P<0.05$). Level of β-tubulin III was not altered. An increase in TH positive cells number was also observed in the presence of vitamin D. Total number of dividing cells was significantly reduced from approximately 15% in untreated cells to 10% in vitamin D treated cells ($P<0.05$).
Discussion: This study shows that vitamin D is capable of directly modulating dopamine synthesis via the regulation of its rate-limiting enzyme TH. Studies are ongoing to investigate the mechanism behind this regulation. Our findings may help to explain epidemiological data linking developmental vitamin D deficiency with schizophrenia and vitamin D deficiency with Parkinson's disease.

POS-WED-117

EARLY ENVIRONMENTAL ENRICHMENT CAN PARTIALLY CORRECT ABERRANT RETINOGENICULATE PROJECTIONS IN TEN-M3 KNOCKOUT MICE

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Visual stimulation is crucial in the normal development and maintenance of the visual system. Through the environmental enrichment (EE) paradigm, where increased sensorimotor stimulation is provided to an animal, the impact of abnormal visual development can be ameliorated. Ten-m3 knock-out (KO) mice show impaired vision, with suppressed binocular activity in the primary visual cortex due to an aberrant mapping of ipsilateral retinogeniculate projections. EE has been shown to restore normal binocular activity in these KOs, however, the locus this improvement remains unclear. By intraocular injection of an anterograde neuronal tracer, the projections from each retina to the dorsolateral geniculate nuclei (dLGN) were visualised. The total proportion of ipsilateral terminals in the dLGN of Ten-m3 KO mice EE from birth was not significantly different to age matched controls ($p=0.063$, n=20 sections from 4 animals, Tukey's HSD). The proportion of ipsilateral terminals targeting the ventral region of the dLGN, however, was significantly reduced in mice EE from birth ($p=0.021$, n=20 sections from 4 animals, Tukey's HSD) from standard-housed controls. KOs EE as adults showed no significant difference in either of these measures ($p=0.237$ and $p=0.523$, respectively, n=20 sections from 4 animals, Tukey's HSD). Our results suggest that EE is able to cause a partial remodelling of the aberrant retinogeniculate projections usually found in Ten-m3 KO mice to more closely resemble those of wildtype mice, potentially contributing to a restoration of binocular activity in the primary visual cortex. The capacity for EE induce changes at the level of the retinogeniculate projection is age-dependent.

(250 words)

POS-WED-118

ELECTROPHYSIOLOGY AND GENE EXPRESSION PROFILES OF NEUROGENESIS IN THE ADULT MOUSE MIDBRAIN

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Whether new dopamine (DA) neurones are generated in the adult midbrain is a controversial and important question for developing better treatments for Parkinson's disease (PD). Evidence indicates Nestin-expressing neural precursor cells (NPCs) give rise to midbrain DA neurones, however this needs to be confirmed, and the underlying mechanisms established. In this study Nestin-expressing NPCs in the adult mouse midbrain were permanently labelled with yellow fluorescent protein (YFP) using transgenic Nestin-CreER^{T2} x R26eYFP mice. One-week to 8-months later YFP+ cells were studied using a combination of whole-cell electrophysiology in acutely prepared midbrain slices and single-cell PCR to measure expression of genes regulating DA neurogenesis. We found a range of electrophysiological and gene expression profiles consistent with birth, migration, neuronal and DA neuronal differentiation, and integration by YFP+ cells. Many cells expressed a combination of mature and immature neuronal and DA genes. Also, the ontogenesis of these cells correlated better with their age than their midbrain location. Our findings are consistent with generation of new neurones and DA neurones from a local population of Nestin-expressing NPCs. They indicate these NPCs are located throughout the midbrain and differentiate and integrate locally, rather than migrate long distances to populate midbrain nuclei. They also suggest many of these cells transition through an unusual hybrid mature and immature neuronal phenotype. Further study of adult midbrain Nestin-expressing NPCs may provide information crucial for developing new and better treatments for PD. (233 words).

POS-WED-119

ROLE OF NICOTINIC RECEPTOR SUBTYPES IN THE DEVELOPING ENTERIC NERVOUS SYSTEM OF MICE

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Objective: Acetylcholine activating pentameric nicotinic receptors (nAChR) is the primary mode of fast excitatory synaptic transmission in the mature enteric nervous system (ENS). We previously reported that embryonic enteric neurons display electrical activity, and that maturation of synaptic transmission continues during early postnatal stages. Here, we examined the involvement of nAChR subtypes during ENS development. **Methods:** Wnt1-Cre;R26R-GCaMP3 mice were used in which all neural crest-derived cells express the genetically encoded calcium indicator, GCaMP3. We studied the myenteric plexus of post-natal (P0, P10 and adult) duodenum and embryonic (E12.5 and E14.5) small intestine. Effects of nAChR subtype antagonists on intracellular Ca^{2+} transients ($[Ca^{2+}]$) evoked by DMPP (nAChR agonist) and single pulse focal stimulation were examined via calcium imaging. **Results:** RT-PCR showed expression of α3,4 and β2,4 subunits in E11.5 and adult gut. At all ages examined (including E12.5) neurons exhibited $[Ca^{2+}]_i$ transients to DMPP and electrical stimulation. These responses were hexamethonium (general nAChR antagonist)-sensitive in E14.5 and post-natal mice. In post-natal mice, SR16584 (α3β4 antagonist) significantly reduced the DMPP response. α-Conotoxin MII (α3β2 antagonist) reduced the DMPP response, but the effect was only significant at P10. Dihydro-β-erythroidine (α4β2 antagonist) significantly reduced the DMPP response in adult and P10 mice, but had no effect at P0. All 3 antagonists reduced the electrical stimulation response at all post-natal ages, except the α4β2 antagonist, which was ineffective at P0. **Conclusion:** Cholinergic transmission in the ENS commences early, well before neurally-mediated motility patterns. nAChR subtypes may play different roles at different stages of ENS development. (250 words).

POS-WED-120

OPTIMISATION OF RED / NEAR-INFRARED IRRADIATION THERAPY FOR TREATMENT OF NEUROTRAUMA

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Spinal cord injury (SCI) is exacerbated by secondary degeneration, associated with increased reactive oxygen species (ROS). Red / near-infrared irradiation therapy (R/NIR-IT) delivered by laser or LED improved functional outcomes in a range of CNS injuries, perhaps by reducing oxidative stress. While studies have shown positive outcomes of R/NIR-IT in SCI using lasers, treatment using LEDs has not been reported and may allow more widespread clinical application. However standardization of treatment parameters is required. We hypothesized that intensities and wavelengths of R/NIR-IT delivered by LED could be optimized to reduce ROS *in vitro* and that these optimized treatment parameters would improve function *in vivo* in a rat SCI model.

We observed no effect of R/NIR-IT on ROS production by rat pheochromocytoma (PC12), Müller (rMC1) or mixed retinal cells treated for 3 min with 670nm light at 8.5×10^{-3} – 3.8×10^{-1} J/cm^2 ($p > 0.05$). Similar results were observed following 3 min exposures to 440, 550, 670 or 810nm light emitting 4.9×10^{14} or 1.25×10^{15} photons/cm^2/s ($p > 0.05$). It is likely that organotypic slice culture models of SCI, irradiated for longer periods of time, may be more useful for optimisation of R/NIR-IT*. In vivo* treatment of SCI using R/NIR-IT at 670nm and 830nm emitting 5.33×10^{16} photons/cm^2/s, did not result in significant improvements in functional recovery ($p > 0.05$), however preliminary morphological analysis suggests increased tissue sparing with R/NIR-IT at these wavelengths. R/NIR-IT may prove useful as part of a combinatorial therapy for SCI. (250 words).

POS-WED-121

GLIAL-MEDIATED INTERHEMISPHERIC FUSION IS REQUIRED FOR EARLY FOREBRAIN DEVELOPMENT AND COMMISSURE FORMATION

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In eutherian mammals, early forebrain formation involves the fusion of the two telencephalic hemispheres along the midline axis. Initially, the two hemispheres are only connected along a single hinge point. Thus, interhemispheric fusion is a critical intermediate step in forebrain development that ensures the cellular integration of the two cerebral hemispheres. This process is then crucial for the subsequent development of major forebrain commissures such as the corpus callosum. However, at present, very little is known about the cellular and molecular events that regulate interhemispheric fusion. Here we show that a specialised population of astroglia known as the midline zipper glia mediates the intercalation of the two telencephalic hemispheres in developing mice and humans. We find that this event involves a precisely timed program of astroglial differentiation that is orchestrated by the secreted protein Fgf8. Using *in vivo* loss- and gain-of-function experiments with Fgf8 and its downstream signalling components, we demonstrate that perturbing midline zipper glia differentiation significantly disrupts interhemispheric fusion and corpus callosum development in mice. These data taken together with analyses of human corpus callosum malformations indicate that interhemispheric fusion defects are a primary aetiology of agenesis of the corpus callosum. (229 words)

POS-WED-122

ANALYSING THE ROLE OF 14-3-3ζ IN INTERNEURON DEVELOPMENT

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14-3-3 are a family of highly conserved intracellular proteins that bind to the phosphoserine/threonine sites on target proteins to modulate their localization and/or activity. Within this family, 14-3-3ζ is highly expressed in the brain and hypothesised to play a major role in neurodevelopment. Previously we have shown that 14-3-3ζ KO mice exhibit anatomical and behavioural traits that are associated with hippocampal defects seen in schizophrenia and other neurodevelopmental disorders, including defects in synapse formation and axonal guidance. The neurodevelopmental defects seen in 14-3-3ζ KO mice arise from abnormal cell patterning in the hippocampus leading to misrouting of mossy fibres and abnormal synapse formation. 14-3-3ζ KO mice show abnormal neuronal positioning, disrupted organization of excitatory neurons and disorganization of neurons in the hippocampal region. We have now expanded our previous findings by identifying a novel role for 14-3-3ζ in the formation of parvalbumin positive interneurons. Immunofluorescence staining of parvalbumin shows a significant decrease in the number of interneurons in the visual cortex and hippocampus of adult mice. Our expression analysis further shows that 14-3-3ζ is expressed in migrating interneurons in the medial ganglionic eminence at embryonic day 12.5. Here we present data to address if this deficiency in interneurons arises from aberrant neuronal specification, neuronal migration, neuronal survival, proliferation and / or differentiation. As this deficiency is similar to the interneuron defects identified in the post-mortem brain of schizophrenia samples our analysis further implicates 14-3-3ζ KO mice as a robust model for schizophrenia. (242 words).

POS-WED-123

HETEROZYGOSITY FOR NUCLEAR FACTOR ONE X AFFECTS HIPPOCAMPAL- DEPENDENT BEHAVIOUR IN MICE

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Identification of the genes that regulate the development and subsequent functioning of the hippocampus is pivotal to understanding the role of this cortical structure in learning and memory. One group of genes that has been shown to be critical for the early development of the hippocampus is the Nuclear factor one (Nfi) family, which encodes four site-specific transcription factors, NFIA, NFIB, NFIC and NFIX. In mice lacking Nfia, Nfib or Nfix, aspects of early hippocampal development, including neurogenesis within the dentate gyrus, are delayed. However, due to the perinatal lethality of these mice, it is not clear whether this hippocampal phenotype persists to adulthood and affects hippocampal-dependent behaviour. To address this we examined the hippocampal phenotype of mice heterozygous for Nfix (Nfix (+/-)), which survive to adulthood. We found that Nfix (+/-) mice had reduced expression of NFIX throughout the brain, including the hippocampus, and that early hippocampal development in these mice was disrupted, producing a phenotype intermediate to that of wild-type mice and Nfix(-/-) mice. The abnormal hippocampal morphology of Nfix (+/-) mice persisted to adulthood, and these mice displayed a specific performance deficit in the Morris water maze learning and memory task. These findings demonstrate that the level of Nfix expression during development and within the adult is essential for the function of the hippocampus during learning and memory.

POS-WED-124

CYSTATIN C REGULATES NEURAL STEM OR PROGENITOR CELL PROLIFERATION

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The normal physiological function of the amyloid precursor protein (APP) of Alzheimer's disease (AD) is largely unknown. Our studies have shown neural stem or progenitor cells (NSPCs) derived from APP overexpressing transgenic mice proliferate more rapidly and differentiate into neurons more readily than wild-type cells. In addition, NSPCs from APP knock-out mice proliferate less rapidly and differentiate into neurons less readily than the corresponding wild-type cells. We found that APP-induced proliferation (but not neuronal differentiation) is mediated by the cysteine protease inhibitor cystatin C. To examine whether the effect of cystatin C related to the inhibition of a cysteine protease, we tested the effect of different cysteine protease inhibitors on NSPC proliferation. The cysteine protease inhibitors E- 64 (0.1-1µM) and antipain (0.15-15 µM) were found to promote NSPC proliferation. In contrast, pepstatin A (0.1-1µM), an aspartic protease inhibitor, and chymostatin (0.1-1 µM), a serine protease inhibitor, had no effect on NSPC proliferation. In conclusion, our results suggest that cystatin C stimulates NSPC proliferation by acting as a cysteine protease inhibitor. The identification of this protease may provide new clues about the regulation of NSPC proliferation and the role of APP in this process. (143).

POS-WED-125

B-CATENIN IS REQUIRED FOR MAINTAINING HIPPOCAMPAL MORPHOLOGY DURING THE PERINATAL PERIOD

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In mice, the compact hippocampal primordium is formed during the prenatal stage by early-generated neurons that migrate from the lateral ventricle zone. The molecular mechanisms that maintain the morphology of the hippocampal primordium after its formation remain to be characterized. β-catenin is a key factor of canonical Wnt signaling and also a component of adherens junctions, and previous embryonic deletion studies have demonstrated that β-catenin is required for early development and generation of granule cells. Herein, we report that perinatal deletion of *β-catenin* specifically in postmitotic forebrain neurons leads to severe defects in hippocampal morphology. *β-catenin* expression is deleted from a cluster of hippocampal cells as early as E18, leading to ectopic migration and arrested cellular differentiation. As a result, the pyramidal and granular layers of the hippocampus become disorganized and several ectopic cellular clusters are observed in the hippocampus of *β-catenin* conditional knockout mice. These findings suggest that β-catenin is required for maintaining hippocampal morphology during the perinatal period.

POS-WED-126

EFFECTS OF AGING ON HUMAN OREXIN EXPRESSION IN THE BRAIN; IMPLICATIONS FOR SLEEP REGULATION AND ENERGY METABOLISM

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Purpose: The orexin neuropeptides (OxA and OxB) have prominent roles in sleep regulation and energy metabolism. With increasing age, there are physiological deteriorations of these functions. Previous studies of the effects of aging on orexin have produced inconsistent results in rodents and humans. **Method:** We studied orexinergic expression in the hypothalamus using immunohistochemistry for OxA and OxB. Three separate age groups included infants (0-1 year) (n=7), children (2-10 years) (n=8) and adults (48-60 years) (n=7) were investigated. The percentage of positive stained neurons and the regional distribution were quantified. **Results:** The percentage of neurons positive for OxA and OxB reduced with increasing age ($P<0.05$). Specifically, infants and children had substantially more OxA and OxB expressing neurons in the perifornical area, dorsal medial and posterior hypothalamus compared to adults, while no significant changes were seen in the lateral hypothalamus. **Conclusion:** Expression of orexin is lower in adults than in infants and children in a region- specific manner. Reduced expression in orexin occurred in the medial and not the lateral fields of the hypothalamus. Insomnia and reduced appetite in late adulthood may be linked to these changes in orexin expression.

(word count: 244)

POS-WED-127

OPTIMISATION OF METHODS TO IMAGE BRAIN VASCULATURE AND VASCULAR REMODELING AFTER PHOTO THROMBOTIC STROKE

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Neural plasticity is recognised to be a critical determinant of good functional recovery following brain injury. This knowledge has driven an intense effort to develop improved neurorestorative interventions that target and enhance this plasticity. Interestingly, there have been comparatively few studies that have examined how the vasculature supports neuroplasticity. To address this issue we were particularly interested in evaluating a variety of established labeling procedures including intra-vital labeling (DiI; FITC-Dextran; Lycopersicon esculentum) and immunohistochemical methods (CD31; eNOS; Laminin; Acquaporin-4 and GSIB4) to identify the vasculature. The objective was to identify labeling protocols that would allow quantitative assessments of vascular alteration and permit the creation of high-resolution digital reconstructions. To induce brain injury we used a model known as photothrombotic stroke. This involves injection of a photoreactive dye to the anaesthetized animal and the subsequent application of a cold light source onto the exposed skull over the cortical region of interest. Light exposure irritates the endothelium resulting in clot formation and occlusion of blood vessels. We identified highly significant differences in the quality of labeling obtained by each of the evaluated methods with DiI producing excellent results, however, dye leakage placed serious time limitations on subsequent imaging. Many of the immunohistochemical methods identified the vasculature but also labeled non-vascular structures limiting their utility for high throughput analyses. In conclusion it appears that separate labeling procedures are likely to be required depending on whether the ultimate objective is the creation of digital models of the vasculature or quantitative analyses of labeling density. (250 words)

POS-WED-128

THE ROLE OF EXTRINSIC VS PRE-PROGRAMMED INTRINSIC SIGNALS DURING NEURAL DEVELOPMENT IN THE ZEBRAFISH RETINA

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During neurogenesis, co-ordinated gene expression acts intrinsically in developing progenitors to regulate neural fate specification. Recent studies demonstrate that extrinsic cues from surrounding cells also bias neural fate decisions, for example to generate proportionally more of an ablated missing cell type. Different types of neurons are generated sequentially, as the gene expression profile within proliferating progenitors changes during development, to drive different fates in differentiating neurons. Whether extrinsic factors influence the progression of these neural fates changes by affecting the cell cycle or intrinsic gene expression remains unknown.

Here, we used morpholino knockdown of the Pancreas transcription factor 1a (Ptf1a), which we showed to be necessary for generating inhibitory neurons, to generate an extrinsic environment lacking these neurons. These inhibitory neurons are usually born at an intermediate stage of retinogenesis.

We assessed whether the loss of these neurons would differentially affect the birth dates and gene expression of later born neurons. Birth dating of retinal neurons was compared in wild type and Ptf1a morphant embryos using prolonged Bromodeoxyuridine pulses every 6 hours from 30 to 66 hours postfertilisation, spanning the entire period of retinogenesis in the central retina. The onset of expression of intrinsic fate determinants was quantified using *in vivo* time-lapse imaging of transgenic reporter lines. Our results show that an environment lacking inhibitory neuron does not change birth dates nor influence the timing of gene expression. We conclude that cell cycle progression and exit, as well as gene expression are solely regulated intrinsically within developing retinal progenitors. (249 words)

POS-WED-129

PROTEIN ARGININE METHYLATION OF TUBULIN ALPHA AND BETA IN MOUSE NEURONS

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Posttranslational modifications are common processes by which eukaryotic cells modify and regulate protein function. Protein-arginine methylation is a common post-translational modification where methyl groups are added to the arginine residues of peptide chains. Protein-arginine methylation plays an important role in many cellular functions both in the nucleus and the cytoplasm such as the regulation of signal transduction, RNA export, and cell proliferation. Tubulin, the building block for microtubules in cells, is known to undergo multiple posttranslational modifications such as detyrosination on the tubulin-α subunit as well as glycylation/glutamylation and acetylation on both tubulin-α and -β subunits. These modifications influence microtubule stability and motor protein guidance. Tubulin is not known to be post-translationally modified by protein methylation.

Here we report that both tubulin-α and -β subunits are methylated on arginine residues. Asymmetric di-methylation on arginine residues was detected by immunoprecipitation and Western blotting. The methylation sites were determined using mass spectrometry.

The results identified two methylated arginine residues each on α-tubulin (R79 di-methylated and R390 mono-methylated), and β-tubulin (R62 and R282 both mono-methylated). The location of these methylated arginine residues on tubulin α and β subunits suggests a potential role in microtubule stability as well as protein-protein interactions with microtubule-associated proteins. Importantly, R282 is involved in binding to microtubule stabilizing drugs such as taxol derivatives. Mutants of R282 are associated with resistance to anti-cancer drugs. Hence our findings have implications for diseases including cancer and drug treatment. (246 words).

POS-WED-130

SEROTONERGIC MODULATION OF NEUROTRANSMITTER RELEASE IN LAYER II/III PYRAMIDAL NEURONS IN RAT SOMATOSENSORY CORTEX

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Somatosensory cortex is innervated by the serotonergic system, but how serotonin (5-HT) modulates transmitter release is poorly understood. **Purpose:** To investigate if and how 5-HT modulates neurotransmitter release. **Methods:** Recordings were obtained from 300µm thick parasagittal slices (P15-19). Miniature excitatory postsynaptic currents (mEPSCs) were recorded from pyramids at 36±1°C in the presence of 1µM TTX and 3µM gabazine at 3-4ml/min. For paired recordings between pyramids, the pre- and postsynaptic cells were current- and voltage-clamped, respectively. Presynaptic action potentials were evoked at 0.2Hz using current steps of ~1.5nA@5ms. **Results:** We found that 10µM 5-HT within the first 5min of its application increased mEPSC frequency transiently by 20±5% from 50±3 to 60±4 Hz (*n*=12), without altering amplitude. Increasing 5-HT to 100µM did not change the outcome (*n*=5). This increase varies with age as it remained the same in P9-12 (21±4% from 35±2 to 42±3Hz, *n*=7), but none was observed in P34-38 rats (*n*=7). In the absence of TTX, 50 µM 5-HT not only increased sEPSC frequency but also its amplitude by 54±30% from -10±1 to -15±3 pA (*n*=3) transiently, indicating involvement of TTX-sensitive conductance. Evoked EPSCs between pairs, at 10µM 5-HT showed a reduction in amplitude by 40±6 % from 9±2 to 5±1 pA (*n*=4) independent of the concomitant presynaptic hyperpolarization and if inhibition was blocked. **Conclusion:** 5-HT modulates transmitter release by two actions in layer II/III of cerebral cortex: an increase of mEPSC together with a depression of evoked EPSCs. Its similarity with noradrenaline may suggest a general principle for neuromodulation.
(250 words).

POS-WED-131

PICK1 INTERACTS WITH PACSIN TO REGULATE AMPA RECEPTOR INTERNALISATION AND CEREBELLAR LONG-TERM DEPRESSION

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The dynamic trafficking of AMPA receptors (AMPARs) into and out of synapses is crucial for synaptic transmission, plasticity, learning and memory. The protein interacting with C-kinase 1 (PICK1) directly interacts with GluA2/3 subunits of the AMPARs. While the role of PICK1 in regulating AMPAR trafficking and multiple forms of synaptic plasticity is known, the exact molecular mechanisms underlying this process remain unclear. Here, we report a novel interaction between PICK1 and all three members of the protein kinase C and casein kinase II substrate in neurons (PACSIN) family and show that they form a complex with AMPARs. Our results reveal that knockdown of the neuronal-specific protein, PACSIN1, leads to a significant reduction in AMPAR internalization following the activation of NMDA receptors in hippocampal neurons. The interaction between PICK1 and PACSIN1 is regulated by PACSIN1 phosphorylation within the variable region and is required for AMPAR endocytosis. Similarly, the binding of PICK1 to the ubiquitously expressed PACSIN2 is also regulated by the homologous phosphorylation sites within the PACSIN2 variable region. Genetic deletion of PACSIN2, which is highly expressed in Purkinje cells, eliminates cerebellar long-term depression (LTD). This deficit can be fully rescued by overexpressing wild-type PACSIN2, but not by a PACSIN2 phosphomimetic mutant, which does not bind PICK1 efficiently. Taken together, our data demonstrate that the interaction of PICK1 and PACSIN is required for the activity-dependent internalization of AMPARs and for the expression of LTD in the cerebellum.

POS-WED-132

BEHAVIOURAL ANALYSIS GASTROINTESTINAL FUNCTION OF THE NL3^{R451C} MOUSE MODEL OF AUTISM

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Patients with Autism spectrum disorder (ASD) commonly display gastrointestinal (GI) problems such as chronic constipation, pain and diarrhea together with clinically recognised psychological traits such as elevated stress and anxiety. Stressful stimuli can increase colonic motility: interconnected brain-gut neural pathways are activated during stress and are relevant to stress-induced gut dysfunction. It is still unclear whether altered GI function in ASD patients originates from Central nervous system (CNS) mediated mechanisms or is intrinsic to the GI system. We aimed to examine stress as a contributing factor to GI disturbances in the NL3^{R451C} mouse model of autism, which express a mutation replacing the arginine at position 451 of neuroligin-3, a synaptic protein, with a cysteine. This mutation was identified in ASD patients and results in altered CNS neurophysiology. Whether NL3 mice display GI phenotypes *in vivo* has not been investigated. We determined the number and weight of fecal pellets produced by male NL3^{R451C} and wildtype littermates when isolated for 1 hour weekly from 4 to 9 weeks of age. After week 1, NL3^{R451C} mice produced significantly more pellets ($P < 0.0001$) and these weighed more ($P < 0.001$) than wildtypes, despite being housed together between collection periods. Ten-week-old NL3^{R451C} mice weighed less than wildtypes. We also examined immunoreactivity for neuroligin-3 in wildtype colon; this is localized to myenteric neuron cell bodies and varicosities. This is the first demonstration of altered GI function in a mouse with an autism producing mutation in a synaptic structural protein.

(246 words)

POS-WED-133

IMPACT OF SK CHANNELS ON CORTICAL EXCITABILITY IN VIVO

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1. Eccles Institute of Neuroscience, Australian National University, Canberra SK channels influence the excitability of cortical pyramidal neurons in complex ways, depending on their subcellular location and how they interact with dendritic calcium and NMDA spikes. We have previously characterised these interactions in acute brain slices, but the diversity of effects observed makes it difficult to predict how SK channels will impact on neuronal excitability in the intact brain. In this study, we used in vivo patch-clamp recordings from cortical layer 5 pyramidal cells in the somatosensory cortex of urethane anesthetized rats in order to investigate the effect of SK channels on neuronal excitability in vivo. Under these conditions, prominent up- and down-states could be observed. While extracellular application of the SK channel blocker apamin revealed no changes in overall firing frequency, and did not influence the occurrence or characteristics of up- and down-states, the distribution of APs within an up-state shifted from an initial burst of APs to a more distributed firing pattern. To quantify this we compared the number of APs within the first 200 ms of an up-state to that during the subsequent 200 ms before (control: 1.517 ± 0.19) and after topical application of a high concentration of apamin (100 µM) to the surface of the cortex (apamin: 1.014 ± 0.23, $p < 0.05$, n=12). These results are consistent with our earlier work in vitro, which showed that blocking SK channel activity increases somatic excitability in cortical pyramidal neurons, but reduces the occurrence of dendritic calcium spikes and associated somatic burst firing.

(247 words)

POS-WED-134

DECIPHERING INTRACORTICAL CIRCUITS IN THE ANTERIOR PIRIFORM CORTEX USING OPTOGENETICS

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We previously described a transgenic mouse line (48L) in which selective expression of a tetracycline-dependent transactivator (tTA-mCitrine) allows us to drive expression of channelrhodpsin-2 (ChR2) in a subpopulation of semilunar (SL) cells in the primary olfactory (piriform) cortex. Here we establish ChR2 expression in 48L mice, and use this system to map some of the excitatory and inhibitory circuits in the piriform cortex. Adeno-associated virus (200 nl) containing a ChR2 construct (TRE-ChR2-mCherry) was stereotaxically injected into newborn 48L mice. After >20 days, 300 µm thick slices of the piriform cortex were prepared and whole-cell recordings were made from histologically-verified neurons at 32 ± 1 o

C. GABAergic interneuron subtypes were identified as previously described (Suzuki & Bekkers, *Cereb Cortex* 20: 2971-2984, 2010). In virus-injected animals (*n*=42), ChR2 expression was restricted to mCitrine-positive (*i.e.* SL) neurons. Photostimulation reliably produced large inward currents (145 ± 48 pA, *n=12)* in ChR2-positive SL neurons, but no response in ChR2-negative SL neurons (*n*=85). In contrast, photostimulation produced inward currents in 17 % of layer 2 superficial pyramidal (SP) neurons (-9 ± 3 pA, *n=181* tested). Photostimulation responses from three different classes of GABAergic interneurons in layer 3 were also measured. Fast-spiking multipolar cells produced the largest response (-77 ± 20 pA, *n=12),* followed by neurogliaform cells (-46 ± 20 pA, *n=14)* and regular-spiking multipolar cells (-6 ± 2 pA, *n=3).* Our experiments indicate that SL cells excite SP cells and layer 3 interneurons, but not other SL cells, confirming less-direct evidence from our previous work. (249 words).

POS-WED-135

MODELING EXCITOTOXIC BRAIN INJURY OF CEREBELLAR PURKINJE NEURONS IN VITRO

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The analysis of cell morphology and connectivity of nerve cells is of particular interest for the study of cellular damage in the central nervous system caused by stroke or neurodegenerative pathologies. Several experimental models have been established to study structural changes under these conditions both *in vitro* and *in vivo*. In this context, the use of transgenic reporter mice has revolutionized the study of neuronal cytoarchitecture. Here, we provide *in vitro* analysis of structural changes of altered complexity of the dendritic arbor of Purkinje neurons in response to excitotoxicity. Cerebellum brain slices from 4-6 week old GAD67-GFP mice, were exposed to 100 µM kainic acid resulting in the increased diameter of dendrites at 10 mins post-administration. This deterioration in Purkinje dendritic arbor continues over time, and by 60 mins, the increase in soma was also evident. When reperfused with oxygenated-artificial cerebrospinal fluid, following 45 mins of excitotoxicity, the swelling and blebbing was reversed. To evaluate the loss of complexity and degradation of the dendrites, image analysis was carried out with Workspace Image Analysis software (CSIRO; Australia), which identified and counted the number of dendrites, length of the dendrites, and mean pixel intensity of the dendrites. Significant differences were observed between pre-excitotoxicity and 30 min ($p<0.01$) or 60 min ($p\leq0.05$) excitotoxicity for number of dendrites and length of the dendrites. Our results highlight a novel method of exploring and analysing the effect of excitotoxicity not only in terms of neuronal viability but also in terms of cytoarchitecture and hence neuronal interactions. (250 words).

POS-WED-136

THE ACTIN CYTOSKELETON REGULATES EXCITOTOXICITY IN THE MOUSE

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Regulation of the cytoskeleton in the postsynaptic compartment of synapses in the nervous system is pivotal for synapse maturation and function. The actin cytoskeleton is the major cytoskeletal structure at the postsynaptic site. A major regulator of the actin filament system is the actin associated-protein tropomyosin. Neurons express products from three tropomyosin genes (*TPM1*, *TPM3* and *TPM4*). We have previously shown that overexpression of the major *TPM3* gene product Tm5NM1 leads to an increase in filamentous actin in neuronal growth cones of cultured hippocampal neurons and that it is enriched at the postsynaptic site of mature neurons in culture. Here, we studied the excitatory phenotype of mice overexpressing Tm5NM1. Continuous EEG recordings of these mice revealed spontaneously occurring seizures which are absent in control mice. Furthermore, the transgenic mice showed higher susceptibility to the seizure-inducing drug pentylenetetrazole. Transcriptome sequencing using hippocampal tissue from transgenic and control mice showed altered expression of genes involved in calcium signalling and ion transport. Altered expression of these genes may contribute to the generation of the seizures which are observed in these mice. Taken together, our data suggest an important functional role for the actin-associated protein tropomyosin at central nervous system synapses.

POS-WED-137

PHARMACOLOGICAL PRECONDITIONING WITH GYKI-52466 IN A KAINIC-ACID SEIZURE MODEL: THE ROLE OF NA^+K^+-ATPASE MODULATION

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GYKI-52466 has been previously demonstrated as a pharmacological preconditioning agent in acute rat models of seizure and stroke (Goulton et al, 2010; Nayak et al, 2012). *In vitro* preconditioning increased Na^+K^+-ATPase (NKA) activity, which may indicate a mechanism allowing neurons to resist excitotoxic depolarization (Goulton et al, 2012). Here we used an established paradigm of low dose GYKI (3mg/kg) to examine whether GYKI administered 90 min prior to a kainic acid (10mg/kg) seizure challenge alters NKA function.

Seizure behaviours were scored to compare KA-alone or GYKI-preconditioned KA-treated animals. Wet-dog shakes, a high-level behaviour characteristic of KA seizures, were significantly reduced ($p<0.05$). Cortical and hippocampal homogenates from these animals, and from animals treated with either saline or GYKI-alone were collected. NKA activity assay revealed increased activity in GYKI-preconditioned KA-treated animals (cortex, $p<0.05$; hippocampus, $p<0.001$), which was not seen following KA-alone or GYKI-alone. Dephosphorylation of the NKAα Ser943 site has been associated with increased NKA activity. Western blotting indicated decreased Ser943 phosphorylation in GYKI-preconditioned KA-treated animals, which was significant in the hippocampus ($p<0.05$) but not the cortex. In addition, an assay of biotinylated surface proteins indicated no change in the levels of NKAα membrane expression.

The current findings suggest that NKA modulation may play a role in GYKI-preconditioning protection, however an insult is required to uncover activity changes. The mechanism behind the activity increase is likely to involve an acute dephosphorylation of the Ser943 site on the α-subunit.

(Word count: 236)

POS-WED-138

DIFFERENTIAL RESPONSE OF CALRETININ-EXPRESSING NEURONS IN THE SPINAL DORSAL HORN TO NEUROMODULATORS

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Neurons in the dorsal horn receive and process a range of noxious and innocuous peripheral inputs. One barrier to understanding how these diverse functions are achieved has been the region's neuronal heterogeneity. We have recently shown that calretinin-expression identifies two distinct populations of neurons: a large population of excitatory interneurons and a smaller population of inhibitory interneurons. In this study, we ask how do these two populations respond to a range of neuromodulators known to influence sensory processing in the spinal cord? Transgenic mice (n =23) expressing green fluorescent protein (GFP) under a calretinin (CR) promoter were anesthetised (ketamine 100mg/kg) and parasagittal spinal cord slices (250µm) were prepared. Patch clamp recordings were obtained from excitatory and inhibitory CR-GFP neurons and responses were assessed during bath application of neuromodulators: noradrenaline (20µM); serotonin (10µM); enkephalin (10µM); and somatostatin (2µM). When neuromodulator responses were detected they appeared as sustained outward, presumptive potassium current, irrespective of the neuromodulator examined. These currents always returned to baseline following removal of the neuromodulator from the bathing solution. CR-GFP positive excitatory interneurons responded robustly to noradrenaline (35/37) and serotonin (18/20), but did not respond to enkephalin (0/20) or somatostatin (0/18). In contrast, CR-GFP inhibitory interneurons did not respond to noradrenaline (0/8) or serotonin (0/4), but responded robustly to enkephalin (6/6) and somatostatin (9/9). Together, these results highlight the differential actions of neuromodulators on spinal sensory processing circuits and support our assertion that excitatory and inhibitory CR-GFP populations represent functionally discrete subpopulations. (243 words).

POS-WED-139

PULSED MAGNETIC FIELDS MODULATE NEURONAL PRIMARY METABOLITES IN VITRO

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Background

Pulsed Magnetic Fields (PMFs) are currently being used to treat a range of neurological conditions such as depression. However, little is known about the cellular mechanisms behind their therapeutic effects.

Objective

To investigate the influence of PMFs on neuronal biochemical processes.

Method

B50 rat neuroblastoma cells were seeded on to 6-well plates, grown to confluence and stimulated with PMFs (~10 mT) at 1 or 10 Hz for 10 minutes (controls were unstimulated). Cells were immediately quenched post-stimulation with ice-cold PBS and then freeze-dried. Cells were subsequently lysed, derivatised and analysed using untargeted gas chromatography-mass spectrometry (GC-MS).

Key Findings

Initial principal component analysis revealed 3 distinct groups (control, 1 and 10 Hz). Significant differences were found in 12 metabolites (PMF stimulated vs unstimulated controls, ANOVA, n= 4-6). PMF stimulated cells had significantly lower levels ($p \leq 0.05$) of GABA precursors (succinate, aspartate, proline and glutamate). These changes may be due to increased inhibitory neurotransmitter release during PMF stimulation. Two metabolites involved in calcium signaling (inositol and serine) were also significantly lower in PMF treated cells. In addition, PMF stimulation at 1Hz reduced metabolite levels to a greater extent than 10 Hz, an effect which reached significance for glycine ($p \leq 0.05$).

Conclusion

PMFs at either 1 or 10 Hz significantly reduced intracellular metabolites involved with inhibitory neurotransmission and calcium signaling in neuronal cell cultures. The changes to neuronal metabolism were frequency dependent. Our data suggest that acute PMF stimulation induce biochemical changes that may modulate inhibitory neurotransmission and calcium signaling.

(246 words)

POS-WED-140

THE PEPTIDE CART EXCITES VASOMOTOR SYMPATHETIC NEURONS

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Hypertension is a major health problem within the Australian population. Sympathetic vasomotor tone is a major determinant in blood pressure and plays a key role in cardiovascular homeostasis. Some current therapeutics are aimed at reducing sympathetic activity, but are non-specific, and thus have wide ranging side effects. Thus, specifically targeting sympathetic vasomotor pathways could lead to new therapeutic avenues. We have recently shown that the peptide cocaine and amphetamine related transcript (CART) is located specifically in preganglionic terminals around sympathetic vasomotor neurons. Therefore, the aim of this study is to characterise the cellular effects of CART on sympathetic vasomotor neurons.
Stellate ganglia were removed from anaesthetised adult Sprague-Dawley rats and acutely dissociated. Standard whole-cell patch clamp recordings were then made from sympathetic vasomotor neurons (retrogradely labelled with Fast Blue from forelimb muscle) and from unlabelled stellate ganglion cells.
Superfusion of CART (1µM) onto 5 retrogradely labelled neurons resulted in a mean depolarisation of 7mV from a mean (± SEM) resting potential of -67.8 (± 1.9) mV to a mean potential of -60.8 (± 2.9) mV after superfusion of CART. In one case the cell started firing spontaneous action potentials.
These data demonstrate that CART specifically excites vasomotor neurons, and thus increase vasomotor tone. This result is at odds with what is currently known of the cellular pharmacology of CART, and suggests that the peptide CART may act on more than one receptor type. (233~~2~~24 words)

POS-WED-141

ACTION POTENTIAL INDUCED SUPPRESSION OF EPSPS BY SK CHANNELS IN DENDRITES

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Calcium plays a pivotal role in the regulation of neuronal function. One way neurons regulate calcium influx is by activation of small-conductance calcium-activated potassium channels, or SK channels. While SK channels have been long known to mediate the medium after-hyperpolarization (mAHP) at the soma, recent studies indicate they can also constrain NMDA receptor activation in dendritic spines during EPSPs. We have previously shown that SK channels in dendrites and spines are activated by backpropagating action potentials (bAPs). Here we investigate the impact of SK channel activation during APs on EPSPs. Somatic whole-cell current-clamp recordings were made from synaptically connected layer 5 pyramidal neurons in brain slices from Wistar rat somatosensory cortex. EPSPs evoked 10 and 20 milliseconds after APs were significantly suppressed compared to control in an apamin-sensitive manner (by ~30% and ~20%, respectively; $n=6$, $p<0.01$). Perturbation of the mAHP by low concentrations of the calcium chelator BAPTA did not affect AP-evoked EPSP suppression ($n=6$). Furthermore, artificial EPSPs generated by somatic current injection were not depressed by SK channel activation during APs ($n=6$). These experiments argue against a role of somatic SK channels in EPSP suppression. Similar results were obtained in layer 2/3 and hippocampal CA1 pyramidal neurons. We hypothesize that during bAPs dendritic SK channels shunt EPSPs, possibly in the spine head. This shunting mechanism is likely to influence calcium influx during co-incident AP firing and synaptic input and may therefore impact on spike-timing dependent synaptic plasticity. (238 words).

POS-WED-142

PAIN INDUCED SYNAPTIC PLASTICITY IN THE AMYGDALA

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Acute pain provides important warnings about dangers in our environment. However some clinical conditions produce persistent pain that outlasts the nociceptive stimuli. The persistence of pain beyond the nociceptive stimulus suggests there are plastic changes in pain pathways that remain after the nociceptive stimulus has stopped. One synaptic pathway that is critical for persistent pain is the spino-parabrachial-amygdala pathway. This pathway delivers nociceptive information to the laterocapsular region of central nucleus of the amygdala (CELC). This project investigated how a brief nociceptive stimulus changes the synaptic properties of the parabranchial (PB) inputs to CELC neurons as these changes are likely to be representative of the initial synaptic changes that occur in development of persistent pain. We used whole-cell patch clamp to define the synaptic properties of the PB-CELC synapse in acute brain slices taken from male Sprague-Dawley rats (3-6 weeks old) that have undergone a brief nociceptive stimulus (44^0C heat for 2 minutes). We found that the brief nociceptive stimulus potentiates PB-CELC synapses through increased AMPA/NMDA ratios. The AMPA/NMDA ratio was maximal 1 day after the nociceptive stimulus (14.4 ± 3.1, n=15 vs. control (33^0C) 4.5 ± 0.6, n=15, p=0.026) and persisted for 3 days (10.2 ±1.2, n=15 vs. control 4.8±1.03, n=7, p=0.043) This data shows that a brief nociceptive stimulus potentiates a synapse that is critical in the development of persistent pain. A better understanding of the mechanisms behind this potentiation may provide a potential drug target in the treatment of persistent pain. (245 words)

POS-WED-143

CLOSTRIDIUM DIFFICILE TOXIN A AND GABA INCREASE THE EXCITABILITY OF MYENTERIC INTRINSIC SENSORY NEURONS IN THE GUINEA-PIG JEJUNUM.

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The enterotoxin (toxin A, TcdA) of the bacteria *Clostridium difficile* is capable of inducing diarrhoea that is life threatening if left untreated. TcdA produces a strong mucosal inflammation that depends on extrinsic primary afferents, but the excess secretion that it produces can be predicted to be mediated, in part, by activation of the enteric nervous system located within the wall of the gastrointestinal tract. However, it is not known how TcdA affects the enteric neural circuitry and whether its actions are comparable to those of exotoxins produced by other diarrhoea-causing bacteria. Additionally, specific bacterial metabolites such as GABA may exacerbate and be associated with persistent *Clostridium difficile* infection. We investigated the effects of TcdA and GABA on the sensory component of the neuronal circuit in the guinea-pig jejunum, using conventional intracellular recordings. We found that pre-treatment with TcdA (12.5µg/ml) in the lumen of isolated jejunum induces hyperexcitability in myenteric sensory neurons shown by an increased number of action potentials (APs) firing during depolarizing current pulses (TcdA: 8.1 ±1.0 APs at 300pA; control: 2.9 ± 0.3 APs at 300pA).GABA (100µM) pre-treatment also made myenteric sensory neurons hyperexcitable (GABA: 4.4 ± 0.8 APs at 300pA; control 2.9 ± 0.3 APs at 300pA). These data parallel our observation that cholera toxin increases excitability of myenteric sensory neurons suggesting that the diarrhoea-producing toxins operate through similar neural circuits. (224 words).

POS-WED-144

PULSED MAGNETIC FIELDS AFFECT ASTROCYTIC GLUTAMATE TRANSPORTERS IN A FREQUENCY-DEPENDENT MANNER

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Glutamate transporters (GLAST and GLT-1) expressed on astrocytes are a primary mechanism for glutamate uptake and recycling in the central nervous system. Astrocytes are responsive to neuronal activity and are capable of releasing neuro-active substances. Astrocytic processes are found immediately adjacent to all glutamatergic synapses and are responsible for maintaining the extracellular space for optimal neuronal function. Astrocytes are therefore a vital component in efficient synaptic communication and a prime target for modulating synaptic plasticity. Pulsed magnetic fields in the form of repetitive transcranial magnetic stimulation (rTMS) are an emerging therapy for brain injury and are generally thought to modulate synaptic plasticity by changing neuronal excitability. However there are very little data on whether rTMS also affects astrocytic function. In this project, we investigated the effect of rTMS (delivered in vitro as repetitive magnetic stimulation - rMS) of different frequencies on glutamate transporter expression in cultured astrocytes. Confluent cultures of neonatal mouse cortical astrocytes were exposed to sham, 1Hz and 10Hz rMS over a 10-minute period. Three hours after stimulation, RNA was extracted and qPCR performed to determine expression of GLT-1 and GLAST genes. 1Hz stimulation had little effect on the genes of interest. 10Hz stimulation resulted in trend towards increased GLT1 expression ($p = 0.18$) and reduced GLAST gene expression. The reduction in GLAST gene expression was accompanied by reduced GLAST protein expression at 24 hours, as assessed by immunohistochemistry. These data support the proposal that rTMS can modulate synaptic plasticity by altering glial as well as neuronal function. (250 words).

POS-WED-145

ASTROCYTES IN TDP-43 PATHOLOGY

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The TAR DNA binding protein TDP-43 is an aetiological factor in motor neuron disease. Pathology is triggered by abnormal processing and cytosolic aggregation of the protein or by mutations in the TDP-43 gene. The pathology is similar and the outcome is directly linked to cell death. TDP-43 pathology is also found in astrocytes: a cell type that plays critical roles in the pathology of MND.

We have established cellular models of TDP-43 proteinopathies by expressing mutant TDP-43 in astrocytes. We have also analysed spinal cord tissue from a transgenic mouse strain expressing the mutant Ala315Thr *TARDBP* gene driven by the mouse prion promoter (Prp-TDP43^{A315T}) for changes in glia related molecules.

TDP-43 mutations affected proliferation of cultured astrocytes cells in a p53-p21 dependent manner with no evidence of cell death. These cells had decreased expression of GLT-1 glutamate transporters and decreased glutamate uptake, reorganisation of cytoskeleton and impaired wound healing (n=5). Mutant astrocytes also displayed changes in mitochondrial membrane potential and in the intracellular transport of these organelles (n=5).

In transgenic animals there was a progressive change in the astrocytic gap junction protein connexin43 and TDP-43 expression. There was also a significant increase in active Caspase-3, GFAP, CD40, CD40L, Dr6, FAS/FASLG, HSP60, IGFBP5 and TNF-alpha expression compared to age matched wild type animals (n=3).

Our results demonstrate pathophysiological effects of mutant TDP-43 on transient transfected astrocytic cells and a pronounced glial activation and production of inflammatory mediators in the spinal cord of animals carrying the same mutation. (246 words)

POS-WED-146

THE PRO-MYELINATING SIGNALLING KINASES ERK1/2 PHOSPHORYLATE OLIGODENDROCYTE-SPECIFIC TRANSCRIPTION FACTORS.

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The Erk1/2 signalling pathway has recently been identified to promote oligodendrocyte myelination both *in vitro* and *in vivo*. Here we investigate the downstream mechanisms that Erk1/2 utilise to promote myelination. Using *in vitro* myelination assays with OPCs infected to overexpress Erk1 or Erk2, we show that Erk2 is the key MAPK that enhances oligodendrocyte myelination (n=5). Erk2 is known to exert some of its effects through direct transcription factor phosphorylation. Our *in silico* analysis identified potential Erk2 binding domains and phosphorylation sites in the oligodendrocyte-specific transcription factors Olig1 and Olig2. These transcription factors are constitutively expressed in the earliest precursor cells through to mature myelinating oligodendrocytes. They have multiple functions and are absolutely required for oligodendrocyte specification, development and myelination. However, how these distinct functions are regulated, and the role Olig1/2 play in these processes remains unclear. Our co-immunoprecipitation experiments show Erk1/2 and Olig1/2 interact both *in vitro* (n=3) and in the developing mouse brain (n=3). We also show that constitutive activation of the Erk1/2 signalling pathway *in vitro* by overexpression of the Erk1/2 activating kinase MEK, induces Olig1 (n=2) and Olig2 (n=2) phosphorylation. Collectively these data suggest that Erk1/2 bind to and phosphorylate Olig1/2 both *in vitro* and *in vivo.* We propose that Erk mediated phosphorylation of Olig1/2 is a key mechanism that switches Olig1/2 to a pro-myelinating function. These data contribute to our understanding of how transcription factors can play distinct roles in cell types with very diverse phenotypes. (241 words)

POS-WED-147

ASSESSING THE ROLE OF GPR62 IN THE MYELINATING OLIGODENDROCYTES OF THE CNS

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The oligodendrocyte is the myelinating glial cell of the central nervous system, responsible for wrapping insulating sheets of myelin around receptive axons, defects of which can cause neurological diseases like multiple sclerosis and leukodystrophies. However, the signalling molecules and receptors that mediate oligodendrocyte development, myelination, and neuron-oligodendrocyte communications remain incompletely understood. We recently identified the orphan G-protein coupled receptor Gpr62 as a highly specific oligodendrocyte transcript, raising the possibility it has a role in myelination or axo-glial interactions. To investigate the role of Gpr62 in vivo we generated a Gpr62 knockout mouse strain. Our initial assessments using transmission electron microscopy and immunohistochemistry showed that the developmental myelin was still intact in the absence of Gpr62. However, using quantitative reverse-transcriptase PCR analysis, we observed significant but subtle alterations in gene expression of select myelin genes when compared to controls in both brain and spinal cord lysates. Our results implicate Gpr62 as a potential regulator of myelin gene expression after post-natal myelination, which could have multiple implications for myelin-plasticity, learning, and multiple sclerosis related therapies. (173 words).

POS-WED-148

ALCOHOL-INDUCED SEDATION AND SYNERGISTIC INTERACTIONS BETWEEN ALCOHOL AND MORPHINE: A KEY MECHANISTIC ROLE FOR TOLL-LIKE RECEPTORS AND MYD88-DEPENDENT SIGNALLING.

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Alcohol increases the risk of opioid-related deaths, due to a pharmacodynamic interaction, which can be quantified in mice as an increase in loss of righting reflex and motor impairment. However, the mechanism of this drug-drug interaction remains elusive. Evidence demonstrates proinflammatory Toll-like receptor (TLR) 2 and TLR4 signalling are induced by morphine, and TLR4 signalling by alcohol. Hence, providing a common site of drug action and a potential novel innate immune- dependent hypothesis for opioid-alcohol drug-drug interactions. Hence, the current study aimed to assess the role of TLR2, TLR4, MyD88 (as a critical TLR-signalling participant), NF-kB, Interleukin-1B (as a downstream proinflammatory effector molecule) and the μ opioid receptor (MOR; as a classical site for morphine action) in acute alcohol-induced sedation (4.5 g/kg) and alcohol (2.5 g/kg) interactions with morphine (5 mg/kg) by assessing the loss of righting reflex (LORR) as a measure of sedation. Wild-type Balb/c mice and matched genetically-deficient TLR2, TLR4, or MyD88 strains were utilized, together with pharmacological manipulation of MOR, NF-kB, TLR4 and Interleukin-1B. Alcohol induced significant LORR in wild-type mice, was halved by MyD88 and TLR4 deficiency, and surprisingly nearly completely eliminated by TLR2-deficiency. In contrast, the interaction between morphine and alcohol was found to be MOR, NF-kB, TLR2 and MyD88 dependent, but did not involve TLR4 or Interleukin-1B. Morphine-alcohol interactions caused acute elevations in microglial cell counts and NF-kB-p65 positive cells in the motor cortex in concordance with wild-type and TLR2 deficient mouse behavioural data, implicating neuroimmunopharmacological signalling in these drug-drug interactions. (246 words)

POS-WED-149

ATTENUATION OF TOLL-LIKE RECEPTOR 4 REDUCES REWARD-LIKE BEHAVIOURS IN MICE

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Alcohol abuse is a significant social and economic problem. Recent evidence suggests alcohol-induced pro-inflammatory central immune signalling may also be involved in the actions of alcohol[1]. Specifically Toll-like receptor 4 (TLR4), a pattern recognition receptor, has found to be essential for many of alcohol actions[1]. The present study aimed to determine whether TLR4 is involved in the rewarding properties of alcohol in mice. The TLR4 signalling pathway was analysed using (+)-naltrexone - a TLR4 antagonist, and genetic TLR4 knockout mice. Reward-like behaviour was assessed using two-bottle choice and conditioned place preference. Control experiments to eliminate confounds of taste were also conducted. Finally, to determine the cell type and which TLR4 signalling pathways were activated in response to alcohol, immunohistochemistry was performed. TLR4 K.O. mice and (+)-naltrexone treated mice demonstrated a reduced preference for consuming alcohol compared to wild-type and saline treated mice respectively (main effect of genotype $p<0.0001$, and (+)-naltrexone $p<0.0001$). However, mice did not differ in saccharin or quinine intake ($p>0.05$). Pre-treatment with (+)-Naltrexone reduced the time spent in alcohol-conditioned chamber compared to saline treated mice in conditioned place preference (two-way ANOVA $p=0.0005$). Genetic and pharmacological TLR4 blockade reduced alcohol preference when assessed by two-bottle choice and conditioned place preference, which is indicative of a reduced reward. This difference was not due to altered taste. Immunohistochemical analysis suggests glia are critical for this response. Collectively, the results suggest that TLR4 contributes to the generation of alcohol-induced reward, suggesting that blocking this signalling may prove beneficial in treating alcohol-abuse disorders. (250 words).

1. Crews, F et al. (2011) Brain Behaviour and Immunity 24 (S1) 4 – 12.

POS-WED-150

CHRONIC CODEINE AND INCREASES IN PAIN SENSITIVITY: MECHANISTIC INSIGHTS AND POTENTIAL TREATMENT STRATEGIES

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Chronic morphine can paradoxically increase pain sensitivity. The widely used opioid codeine is partially metabolised (≈10%) to morphine to elicit analgesia. Chronic morphine exacerbates pain by activating the immune system in a toll-like receptor-4 (TLR4) dependent manner. *In-silico* simulations indicate codeine also docks to TLR4, suggesting potential to induce TLR4-dependent pain enhancement, independent of conversion to morphine. We hypothesise codeine will cause hyperalgesia and allodynia independent of its opioid receptor-dependent analgesic rank potency.

Hyperalgesia and allodynia were assessed using hot-plate and von-Frey tests respectively, on days 0, 3 and 5, in male mice receiving equimolar codeine, morphine 20mg/kg or saline (n=8/group), twice daily (i.p.). Dosing was repeated in mice primed with prior partial nerve injury and in TLR4 knockout mice. Interleukin-1 receptor antagonist (IL-1RA) and ibudilast interventions were employed in additional experiments to elucidate mechanisms involved.

Hot-plate latency (s) was reduced, following codeine (-9.5, CI:-4.7 to -14.3) and morphine (-7.3, CI:-2.3 to -12.3) vs saline at day 5. Prior partial nerve injury induced hyperalgesia sooner (day 3) vs drug alone. TLR4 knockout mice were protected from opioid-induced hyperalgesia and allodynia. Codeine-induced changes in hot-plate latency and paw withdrawals were reversed by L-1RA (8.6, CI:5.1 to12.2 and -14, CI:-20.8 to -6.9 respectively). Ibudilast reversed codeine-induced changes in paw withdrawals (-22, CI:-0.9 to -42.5).

Equimolar codeine and morphine induce similar degrees of TLR4 and interleukin-1-dependent hyperalgesia and allodynia, suggesting codeine does not solely rely upon conversion to morphine to increase pain sensitivity. Findings are consistent with a glial-mediated hypothesis of pain facilitation.

(250 words)

POS-WED-151

CALORIE RESTRICTION ATTENUATES LIPOPOLYSACCHARIDE (LPS)-INDUCED MICROGLIAL ACTIVATION IN DISCRETE REGIONS OF THE HYPOTHALAMUS AND THE SUBFORNICAL ORGAN

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Calorie restriction (CR) has been shown to increase longevity and elicit many health promoting benefits including delaying immunosenescence and attenuating neurodegeneration in animal models of Alzheimer's disease and Parkinson's disease. CR also suppresses microglial activation following cortical injury and aging. We previously demonstrated that CR attenuates lipopolysaccharide (LPS)-induced fever and shifts hypothalamic signaling pathways to an anti-inflammatory bias; however, the effects of CR on LPS-induced microglial activation remain largely unexplored. The current study investigated regional changes in LPS-induced microglial activation in mice exposed to 50% CR for 28 days. Immunohistochemistry was conducted to examine changes in ionized calcium-binding adapter molecule-1 (Iba1), a protein constitutively expressed by microglia, in a total of 27 brain regions involved in immunity, stress, and/or thermoregulation. Exposure to CR attenuated LPS-induced fever, and LPS-induced microglial activation in a subset of regions: the arcuate nucleus (ARC) and ventromedial nucleus of the hypothalamus (VMH) and the subfornical organ (SFO). Microglial activation in the ARC and VMH was positively correlated with body temperature. These data suggest that CR exerts effects on regionally specific populations of microglia; particularly, in appetite-sensing regions of the hypothalamus, and/or regions lacking a complete blood brain barrier, possibly through altered pro- and anti-inflammatory signaling in these regions. (202 words)

POS-WED-152

CHRONIC RESTRAINT STRESS INDUCED REMODELLING OF ASTROCYTES IN THE RAT HIPPOCAMPUS.

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While astrocytes have been implicated in the aetiology of stress induced mood disturbance there specific contribution has remained difficult to determine. For some time it has been recognised that exposure to one of the principal changes produced by stress was a reduction in glial fibrillary acidic protein (GFAP). Initially, this was taken to indicate that exposure to chronic stress caused the death of astrocytes within the brain. Recently, however, stress rather than causing cell death appears to induce significant structural changes within the astrocytes cytoskeleton, at least within the prefrontal cortex. In the current study we wish to examine whether the same phenomena occurred within the hippocampus another brain region that is recognised to be exquisitely sensitive to the effects of chronic stress. We have now found that astrocytes within the hippocampus like the prefrontal cortex also undergo significant structural remodelling as a result of stress. We have further examined how these changes may be linked in with broader disturbances known to be elicited by chronic stress. Specifically we have examined changes in astrocyte specific glutamate transporters and glucocorticoid receptor density. Collectively, these findings highlight that structural remodelling of astrocytes may be phenomena that allows the stressed brain to adapt to chronically stressful environments.

POS-WED-153

SUBSTANCE P PROMOTES THE TRANSLOCATION OF THE δ-OPIOID RECEPTOR ON CHOLINERGIC INTERNEURONS IN THE STRIATUM

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The ∂-opioid receptor (DOR) is a G-protein coupled receptor that is heavily expressed in the striatum, including in cholingeric interneurons (CIs). In these cells, under baseline conditions, large amounts of the receptor are sequestered in intracellular pools and are presumably inactive. Pavlovian conditioning promotes the insertion of the receptor into the somatic membrane of CIs in the nucleus accumbens shell (NAc-sh). However, the molecular mechanism that triggers this translocation event is unknown. The present study investigates whether signalling of the neurotransmitter substance P (SP) in the striatum facilitates this insertion. SP (300nm) was administered unilaterally to the striatum of DOR-eGFP mice *in vivo* for 30 minutes and to slices *in vitro* for 15, 30 and 60 minutes. DOR-eGPF distribution was visualised with immunohistochemistry and DOR-eGFP receptor at the membrane of CIs was quantified with ImageJ. *In vivo,* SP treatment drives a significant increase in the membrane levels of DOR-eGFP in both the dorsal medial striatum (DMS) ($p<0.0001$) and NAc-sh ($p<0.05$). *In vitro*, SP treatment significantly increased the levels of membrane DOR-eGFP at 15 and 30 minutes in the nucleus accumbens core (NAc-co) ($p<0.001$) and at 30 minutes in the NAc-sh ($p<0.01$). Previously, SP has been controversially linked to DOR translocation via a cotrafficking mechanism. Here, we demonstrate that SP may be involved in DOR translocation through an alternative interaction – namely SP signalling. These data demonstrate a novel mechanism of DOR translocation, and a novel role for SP, which may contribute to the behavioural effects of opioids in the striatum. (249 words)

POS-WED-154

THE PHARMACOLOGICAL PROFILE OF LOCAL POSTERIOR HYPOTHALAMIC THETA RHYTHM IN ANESTHETIZED RATS.

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We have just recently demonstrated independent of hippocampal (HPC) theta rhythm, local theta activity in posterior hypothalamic (PH) region (Kowalczyk et al., Hippocampus, 2014). In a present study we emphasize the pharmacological profile of PH theta rhythm and compare the pharmacological properties of PH theta rhythm with theta recorded from HPC.

1. Both HPC and PH theta are cholinergic-mediated: are blocked by atropine sulphate.

2. HPC theta rhythm is blocked by Gap junction (Gj) blocker, carbenoxolone. This effect was antagonized by trimethylamine, Gj opener. In contrast, PH theta was induced by carbenoxolone and this effect was antagonized by spironolactone, blocker of mineralocorticoide receptor.

3. HPC theta rhythm can be induced both by orexine A and B. In contrast, PH theta rhythm has never been induced by orexine A and B.

The results provide an additional evidence that there is in fact autonomous theta field potential in the PH region. The research project was supported by NCN grant no. 2011/01/NZ4/0073.

POS-WED-155

A SELECTIVE OREXIN-1 RECEPTOR ANTAGONIST SB-334867 ATTENUATES RAT ANXIETY DURING CAT ODOUR EXPOSURE.

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Anxiety disorders are the most common type of psychiatric disorders in Australia and cause a wide array of associated symptoms. Recently the neuropeptide orexin has been implicated in mediating anxiety as there are dense orexin neuron projections to the limbic system. Previous research has used orexin receptor antagonism during traditional animal models of anxiety, showing mixed results. We aimed to determine if cat-odour induced anxiety, expressed as behaviour and neuronal activation, could be attenuated by pretreatment with the orexin-1 receptor antagonist SB-334867. Male Wistar rats were assigned to three groups, no odour with vehicle, odour with vehicle, and odour with SB-334867 pretreatment. Rats were administered 10 mg/kg of SB-334867 or vehicle (3 ml/kg) 10 minutes prior to a single exposure to cat odour from a worn cat collar where behavioural measures were recorded for 20 minutes. Brain tissue was subsequently processed for c-Fos immunohistochemistry to quantify brain region activation. Cat odour exposure increased all measured anxiety-like behaviours as well as increased activation in the posteroventral medial amygdala (MePV), paraventricular hypothalamus (PaV) and dorsal premamillary nucleus (PMd). Pretreatment with SB-334867 attenuated the anxiety-like behaviours of approach and motor activity, and reduced Fos expression in the PaV and PMd. These results implicate the orexin systems in mediating anxiety-like behaviours associated with direct contact with predatory stimulus and suggested that the PMd and PaV mediated this response through orexin transmission. (226 words).

POS-WED-156

STEM CELL TRANSPLANTATION IN TRAUMATIC SPINAL CORD INJURY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Stem cell therapy is a candidate for the treatment of spinal cord injury and it is essential that our appraisal is realistic and unbiased. Systematic review, meta-analysis and meta-regression were used to study the impact of stem cells as a therapy in animal models of spinal cord injury. 156 publications reporting 45 different stem cell preparations met our inclusion criteria. Implantation improved motor (effect size 27.3%, 95% CI 24.7 – 29.8%; 318 comparisons) and sensory outcome (Effect size 17.4% [10.9 – 23.8%]; 24 comparisons). For sensory outcome most heterogeneity was accounted for by facets of stem cell biology. An embryonic source, differentiation before implantation and intravenous route of delivery favoured better outcome. Stem cell implantation did not appear to improve sensory outcome in females and appeared to be enhanced by isoflurane. Biological plausibility was supported by a dose response relationship. For motor outcome facets of stem cell biology had little detectable effect, instead most heterogeneity could be explained by the experimental modelling paradigm. The location of injury, method used to induce injury, method of assessment, presence of immunosuppression and choice of anaesthetic all had an impact. In the large and relatively homogeneous portion of the data set provided by rats implanted with allogeneic stem cells after injury created with an impactor at the mid thoracic level and assessed by BBB test, methodology and in particular choice of anaesthetic and use of immunosupression accounted for most of the heterogeneity.

POS-WED-157

BLOCKING 5-HT2A AND 5-HT7 BUT NOT 5-HT2C RECEPTORS IMPAIRS LOCOMOTION IN INTACT RATS.

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It is widely accepted that applying serotonergic agonists or grafting of fetal serotonergic cells into the spinal cord improves locomotion after spinal cord injury. It has been suggested that the recovery of locomotor activity in spinal rats is due to upregulation of constitutively active 5-HT2A receptors. Little is known about the role of 5-HT receptors in the control of voluntary locomotion, so we administered antagonists of 5-HT2A (cyproheptadine), 5-HT2C (SB242084) and 5-HT7 (SB269970) receptors intrathecally in intact rats and monitored their effects on unrestrained locomotion. An intrathecal cannula was introduced at the low thoracic level and pushed caudally until the tip reached the L2 or L5 segment. Locomotor performance was evaluated using EMG activity of hindlimb muscles during unrestrained locomotion on a 2 m long runway or on a treadmill. Stretch reflexes were estimated using EMG recordings during dorsi- and plantar flexion at the ankle. We found that locomotion can be dramatically impaired after the blockage of 5-HT2A and 5-HT7 receptors. Initially, complete hindlimb paralysis was observed, but with persisting EMG activity. Intra and interlimb coordination was also impaired. Cyproheptadine additionally decreased locomotor EMG amplitude and abolished or substantially decreased stretch reflexes. Blocking 5-HT2C receptors had no effect either on locomotion or reflexes. We suggest that in intact rats serotonin controls timing and amplitude of muscle activity by acting on 5-HT2 receptors on both CPG interneurons and motoneurons, while acting on 5-HT7 receptors it influences interneurons involved in producing the locomotor pattern.
Supported by Polish MSHE N N404 318040.(249 words)

POS-WED-158

CAN PRO-INFLAMMATORY CYTOKINE GENE EXPRESSION EXPLAIN MULTIFIDUS MUSCLE FIBRE CHANGES AFTER AN INTERVERTEBRAL DISC LESION?

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Structure and behavior of the multifidus muscle change in back pain, but the mechanisms are poorly understood. Although changes in muscle fibre types have the potential to unify the observations, the effect of injury on muscle fibre distribution has not been adequately tested and understanding of mechanisms is limited. This study investigated the effect of an intervertebral disc (IVD) lesion on the proportion of slow, fast and intermediate muscle fibres in the multifidus muscle in sheep, and whether muscle fibre changes were paralleled by local gene expression of the pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α). The L1-2, L3-4 and L5-6 IVDs of 11 male wether sheep received anterolateral lesions. Six control sheep underwent no surgery. Multifidus muscle tissue was harvested at L4 for muscle fibre analysis using immunohistochemistry, and L2 for cytokine analysis with PCR for local gene expression of TNF-α. The proportion of slow muscle fibres in multifidus was less in the lesioned animals both ipsilateral and contralateral to the lesion. The greatest reduction in slow fibres was in the deep medial muscle region. A greater prevalence of intermediate fibres on the uninjured side implies a delayed fibre type transformation on that side. TNF-α gene expression in multifidus was greater on the side of the lesion than the muscle of control animals. These data provide definitive evidence of muscle fibre changes following induction of an IVD lesion and a parallel increase in TNF-α expression. Pro-inflammatory cytokine changes provide a novel mechanism to explain behavioral and structural changes in multifidus.

(260 words)

POS-WED-159

MICROSTIMULATION OF SINGLE MOTOR AXONS: A COMPARISON OF THE CONTRACTILE RESPONSES TO IRREGULAR AND REGULAR TRAINS OF STIMULI

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Purpose: During voluntary contractions, human motoneurones discharge with a physiological variability of ~25%. However, studies that have measured the contractile responses to microstimulation of single motor axons have used regular trains of stimuli with no variability. We tested the hypothesis that irregular (physiological) trains of stimuli produce greater contractile responses than regular (non-physiological) trains of identical mean frequency but zero variability. **Methods:** High-impedance tungsten microelectrodes were inserted into the common peroneal nerve and guided into fascicles supplying extensor hallucis longus or extensor digitorum longus. Selective microstimulation was achieved for 14 single motor axons. Contractile responses were measured via an angular displacement transducer over the relevant toe. After recording the responses to regular trains of 10 stimuli extending from 2-100 Hz, irregular trains of 10 stimuli – based on the interspike intervals recorded from single motor units during voluntary contractions were delivered. Finally, the stimulation sequences were repeated following a 2 min period of continuous stimulation at 20 Hz to induce muscle fatigue. **Results:** Regular trains of stimuli generated a linear increase in displacement with frequency, whereas irregular trains - emulating the firing of volitionally-driven motoneurones, - displayed significantly greater responses over the same frequency range (8-24 Hz). This was maintained even in the presence of fatigue. **Conclusions:** We conclude that physiological discharge variability, which incorporates short and long interspike-intervals, offers an advantage to the neuromuscular system by allowing motor units to operate on a higher portion of the contraction-frequency curve and taking advantage of catch-like properties in skeletal muscle.

POS-WED-160

MOTOR END PLATE (MEP) TARGETED ADMINISTRATION OF NEUROANATOMICAL TRACER IN THE RAT HINDLIMB ENHANCES RETROGRADE TRANSPORT INTO SPINAL CORD MOTOR NEURONS

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Access to the spinal cord motor neurons innervating hindlimb muscles could be an important tool in therapeutic interventions seeking the recovery of motor function after injury. MEPs are specialised regions on the skeletal musculature that offer minimally invasive access to the pre-synaptic nerve terminals, henceforth to the spinal cord motor neurons. Gene therapy can therefore be performed by utilising the MEPs as a conduit to shuttle therapeutic genes into the ventral horn of the spinal cord. In this study, we demonstrate the efficiency of targeting the entire MEP regions of hindlimb muscles to attain greater uptake into motor neuron cell bodies in the lumbar spinal cord. Whole rat hindlimbs were subjected to acetylcholinesterase histochemistry to reveal the location of the MEPs for targeted muscles. This information was then utilised to guide a series of injections of Fluoro-Gold (FG) into targeted muscles in naïve Long-Evans rats. After such intramuscular injections, the spinal cords were dissected out, sectioned and the tissue was analysed under epifluorescence for the presence of FG-labelled motor neurons. This analysis revealed that MEPs were found to span the entire width of hindlimb muscles. The motor neurons innervating individual hindlimb muscles were observed to exist in columns spanning multiple segments within the spinal cord. These columns of motor neurons overlap along all axes of the cord. These results indicate that enhanced somatic delivery of therapeutic agents into both spinal cord motor neurons and neuromuscular junctions can be achieved by means of MEP-targeted administration.

244 words

POS-WED-161

DISSOCIATION OF THE EFFECT OF LESIONS TO THE PARVOCELLULAR AND MAGNOCELLULAR COMPARTMENTS OF THE RED NUCLEUS (RN) ON SKILLED REACHING: AN ANATOMICAL AND BEHAVIOURAL INVESTIGATION IN THE RAT

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We have shown that rubrospinal tract (RST) lesions in the rat interfere with one of the components of the reach, namely the arpeggio movement. We also showed that complete RN lesions not only interfere with the arpeggio, but also create additional impairments in reaching. Arpeggio deficits were expected after RN lesions as the magnocellular compartment of the RN (mRN) give rise to the RST. Additional impairments after complete RN lesions can be explained in terms of the connectivity of the parvocellular compartment of the RN (pRN) with structures directly involved in motor control such as the motor cortex and the cerebellum. The present study was an attempt to dissociate the roles of the pRN and mRN in skilled reaching. Rats were trained on the skilled reaching task, after which they were subjected to unilateral lesions of either the mRN or the pRN. Post-surgical performance on the skilled reaching task was compared to baseline in the two groups. Lesions to the mRN only gave rise to impairment in arpeggio. This result is in line with anatomical data showing that the origin of the RST lies mainly within the mRN. Lesions over the pRN did not produce a deficit in the arpeggio movement, but such lesions interfered primarily with the ability to guide their forelimb towards the food and to supinate the paw after the reach. This is the first study showing a dissociation of the effect of selective lesions to the pRN and the mRN in skilled reaching in the rat.

Word count: 250

POS-WED-162

INVESTIGATING TASK RELATED CHANGES IN INTRACORTICAL INHIBITION USING PAIRED- AND TRIPLE-PULSE TRANSCRANIAL MAGNETIC STIMULATION

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Recent research has demonstrated variations in activity-related modulation of intracortical inhibition within primary motor cortex (M1) between tasks requiring isolated (abduction) or synergistic (precision grip) muscle activation. The current study sought to investigate task-related changes in pre- and post-synaptic intracortical inhibition in M1.

In 15 young adults (22.5±3.5 years), paired-pulse transcranial magnetic stimulation (TMS) was used to measure short- (SICI) and long-interval intracortical inhibition (LICI) in resting first dorsal interosseous muscle, and during abduction of the index finger or precision grip of the index finger and thumb. Triple-pulse TMS was used to investigate changes in LICI-SICI interactions during these tasks.

SICI was reduced during abduction and precision grip compared with rest, while SICI during precision grip was also less than during abduction. LICI obtained at two interstimulus intervals (ISI, 100 and 150ms) differed between active states; 100ms ISI produced increased inhibition for both abduction and precision grip compared with rest, whereas the 150ms ISI produced decreased inhibition during both abduction and precision grip compared with rest. SICI was reduced in the presence of LICI at 100ms and 150ms ISI's at rest, while reduced inhibition was observed at the 100ms ISI during abduction and there was no change in inhibition at either ISI during precision grip.

Results suggest that synergistic contractions require greater disinhibition of M1 than isolated contractions. They also suggest task-related differences in the contribution of presynaptic mechanisms to this disinhibition. Furthermore, the assessment of LICI using different ISIs may not represent activation of the same cortical process. (249 words)

POS-WED-163

A MULTI-SIMULATOR FRAMEWORK FOR ACCESSIBLE LARGE-SCALE SIMULATIONS OF BIOPHYSICAL NEURONAL NETWORKS

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Detailed computational models of neuronal systems are able to integrate a broad range of experimental data to aid the development and validation of theories that are not obvious to the naked mind. However, the structure and parameters of such models are often obscured by the algorithmic code required to simulate them on high-performance computing (HPC) clusters. The inaccessibility of the model configurations not only prevents a broad audience from being able to properly scrutinise the data and assumptions the models are derived from, but also makes porting the simulations to alternative simulators--providing important validation of their results--labour intensive and error prone.

We have developed an extension to NineML, an XML-based neuronal modelling language, to enable the definition of biologically detailed neuronal network models, along with a multi-simulator pipeline to simulate the models on HPC clusters. With the aid of standard editing software, the XML definitions provide fast, unambiguous access to the complete model configuration. From these definitions, the software pipeline compiles the cellular dynamics into simulator-dependent formats for either the NEST or NEURON simulators, builds the specified neuron populations and projections using the PyNN simulator-independent language, before executing the simulation.

A model of the cerebellar granular layer is implemented in this framework, and comparable simulation results are demonstrated using the NEURON and NEST simulators. Such models can be easily shared and extended, promoting code reuse within the community, which should lead to more reliable, accurate and accessible computational models of neuronal systems. (244 words).

POS-WED-164

EVALUATION OF HEURISTICS FOR ELIMINATING MUSCLE ACTIVITY FROM ELECTROENCEPHALOGRAM

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Cerebral activity measured by scalp sensors is highly susceptible to persistent electromyographic (EMG) contamination, which is always present due to continuous, variable and usually mild contraction of the postural and facial muscles. Independent components analysis (ICA) has proven effective at separating neurogenic from myogenic signals. Correct identification of the origin of the separated independent components (ICs) typically applies a set of heuristics based on known characteristics of the signal types.

We evaluated standard heuristics for identifying EMG. We made scalp recordings on awake subjects in the absence and in the presence of complete neuromuscular blockade. Contamination was quantified by comparing power after paralysis (predominantly brain signal) with power before paralysis (brain plus muscle signal).

There was a substantial reduction in pre-paralysis power across the whole scalp following the removal of heuristically-identified EMG ICs. Centrally, where muscle contamination is least, there is a greater than 35% reduction in power at frequencies 30-200 Hz. Peripherally, where muscle contamination is greatest there is a greater than 85% reduction in power at frequencies 30-200 Hz.

Our results demonstrate that the combination of ICA and heuristics to identify muscle components can result in substantial reductions of muscle contamination and consequently more robust estimates of brain activity measured at the scalp. (209 words)

POS-WED-165

DEVELOPING YEAST AS A SCREENING TOOL FOR IDENTIFYING NOVEL THERAPEUTIC AGENTS FOR ALZHEIMER'S DISEASE

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Drug development in Alzheimer's disease (AD) is driven mainly by the amyloid hypothesis and the beta amyloid protein (Aβ) is a major target for developing therapeutics. Dysfunction of cellular degradation pathways leading to increased accumulation of Aβ in the brain has been implicated in the neurodegenerative process in AD. However, the underlying cellular mechanisms are not well understood and reducing its accumulation and preventing associated neurotoxicity has remained a challenge. Yeasts are simple eukaryotic cells and provide a greater advantage over other models mainly due to its powerful genomic screening methods. In addition to similarity in gene sequences, yeast cells demonstrate similarity in various cell signalling pathways and have been used as a model for neurodegenerative disorders characterized by protein misfolding and accumulation.
Here, we report a novel high-throughput assay based on our previously established GFP-Aβ expression system in yeast for studying clearance of Aβ aggregates. This fluorescence based assay will be utilized to screen GFP-Aβ levels in 146 individual yeast gene deletion mutants lacking specific proteins associated with the core machinery and signalling pathways of autophagy. The genes that regulate Aβ levels identified in the yeast screening study will be further validated using a plasmid overexpression system. Functional analysis of the target genes will be undertaken to determine how they modulate localization and trafficking of the GFP-Aβ protein to enhance its clearance in the cell. Also, this yeast model offers a novel platform for a cost-effective assay that can be utilized in the development of anti-AD drugs targeting Aβ clearance.

POS-WED-166

REGISTERING CORTICAL MAPPING DATA TO THE MARMOSET ATLAS

Chaplin TA[1], Yu HH[1], Majka P[3], Yen CC[4], Bakola S[1], Kowalski JM[3], Hung CC[4], Burman KJ[1], Wójcik DJ[3], Silva AC[4] & Rosa MGP[1,2]

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The growing number of techniques for mapping the cerebral cortex are producing a rich but somewhat disparate view of cortical organization. One potential solution is to consolidate the data by registering to a standardized atlas. Here we examined the registration methods available and their suitability for different types of data in the studies of the marmoset monkey. We have constructed two types of atlas - surface and volumetric versions, each allowing for different types of registration, visualization and analysis. The surface atlas is well suited to visualizing 3D and flattened representations of the cortex and usually offers more accurate registrations but cannot represent cortical layers and subcortical structures. The volumetric atlas overcomes these limitations but is best suited to registering to inherently 3 dimensional data sets such as MRI. We have investigated registering several types of data to the atlas: retrograde tracer injections, microelectrode recordings and in vivo myelin mapping with MRI. Tracer injections and microelectrode recordings datasets are first reconstructed to 3D surface models from 2D histological sections and then registered to the surface atlas, whereas the MRI data is registered to the volumetric atlas. In general, there is good correspondence between the registered data and what the atlas anatomy predicts, but there are some differences. It is unclear at this stage if these differences are mainly due to shortcomings in registration or is perhaps due natural variability between individuals. [231 words]

POS-WED-167

RADIATION INDUCED EXPRESSION OF PLATELET ENDOTHELIAL CELL ADHESION MOLECULE-1 IN ARTERIOVENOUS MALFORMATIONS AND CEREBRAL ENDOTHELIAL CELLS

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Cerebral arteriovenous malformations (AVMs) are congenital vascular abnormalities associated with significant mortality and morbidity. However, conventional treatment paradigms insufficiently address large deeply seated AVMs. The innovative application of radiosurgery followed by vascular targeting for AVM treatment, may overcome this problem by exploiting radiation-induced unique molecule expression on the AVM endothelium, thereby inducing rapid and selective vessel occlusion. Platelet endothelial cell adhesion molecule-1 (PECAM-1) has been shown to upregulate after radiosurgery on the surface of endothelial cells, demonstrating its potential utility as a therapeutic target. However, the post-irradiation expression of PECAM-1 on cerebral endothelial cells and in an AVM animal model remains unexplored. The aim of this study was to quantify the temporal expression of PECAM-1 in 1) cultured bEnd.3 endothelial cells, and 2) an animal model of AVM after radiosurgery.

Genes encoding for PECAM-1 were significantly upregulated post-irradiation ($p<0.05$). Up-regulation could be detected at 6 hours post-irradiation. An over 11-fold increase was detected in gene expression at 120 hours in comparison to non-irradiated controls. In-Cell ELISA demonstrated significant upregulation of surface PECAM-1 on bEnd.3 cells. However, significant PECAM-1 up-regulation following irradiation was not detected in the AVM model, possibly due to the semi-quantitative nature of the detecting method. These data demonstrate that radiosurgery can significantly alter PECAM-1 expression in vitro, potentially enabling the use of ligand-based molecular targeting therapies for AVM treatment. More sensitive and quantitative methods, such as positron emission tomography, may further elucidate PECAM-1's suitability as a vascular target in vivo (243).

POS-WED-168

RADIATION INDUCED GENE EXPRESSION OF VON WILLEBRAND FACTOR, HIGH-MOBILITY GROUP AT-HOOK 2, AND ENDOTHELIAL CELL-SELECTIVE ADHESION MOLECULE IN CEREBRAL ENDOTHELIAL CELLS

Chen B[1,2], Raoufi-Rad N[1], Reddy R[1,2], Zhao Z[1], Lee V[1], Grace M[1], Ukath J[1], Stoodley M[1,2]

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Cerebral arteriovenous malformations (AVMs) are congenital vascular abnormalities associated with significant mortality and morbidity. However, conventional treatment paradigms insufficiently address large deeply seated AVMs. We hypothesise that radiosurgery can induce unique molecule expression on the AVM endothelium, allowing vascular targeting to act more rapidly and selectively on the AVM vessels. The aim of this study is to investigate the gene expression of potential molecular markers post-irradiation on cerebral endothelial cells. A murine brain endothelial cell line (bEnd.3) was treated with 25 gray radiation using a linear accelerator. Non-irradiated cells were used as controls. Real-time quantitative polymerase chain reaction was used to determine the relative gene expression of bEnd.3 cells at 0, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-irradiation. Genes encoding for von Willebrand factor (vWf), high-mobility group AT-hook 2, and endothelial cell-selective adhesion molecule were found to be significantly upregulated post-irradiation at varying time points ($p<0.05$), with the maximum level of gene expression for vWf being evident at 120 hours post-irradiation (over 8 fold increase in comparison to control samples). All molecules demonstrated significant upregulation at 120 hours post-irradiation. These data demonstrate that radiosurgery can significantly alter endothelial cell phenotype in vitro, potentially enabling the use of ligand-based molecular targeting therapies for AVM treatment.

POS-WED-169

TRACKING OF AUTOLOGOUS VSOP-LABELED MESENCHYMAL STEM CELLS IN THE SHEEP BRAIN USING 3T MRI.

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Mesenchymal stem cells (MSC) therapy of stroke was reported efficient in various small animal studies. However, there is still limited knowledge about cell homing towards the lesion. In this study, a sheep model of focal cerebral ischemia was utilized, to assess the detection limit for autologous ovine (o)MSC in the brain, and to track intravenously (i.v.) and intraarterially (i.a.) injected oMSC after stroke.

Six healthy animals received targeted intracerebral injections of VSOP-labeled oMSC (0; 500; 1,000; 10,000; 100,000 cells). Brains were subsequently analyzed by 3.0T clinical MRI. Additional six sheep were subjected to permanent middle cerebral artery occlusion (MCAO). Three subjects each received i.v. or i.a. injection of VSOP-labeled oMSC 24h after MCAO, respectively. Cell tracking by MRI was conducted at day1, day3, and day7 after administration. Histological examination was performed on brain tissue, head wounds, and main organs at day8 after MCAO.

1,000 oMSC were reliably detectable at 3.0T. However, no labeled cells were identified within the sheep brain after i.v. injection. After i.a. injection, a signal loss on MRI and iron-positive cells in brain parenchyma were detected in one subject. In contrast, iron-labeled cells were histologically detected in skin wounds, lung and spleen in all animals.

Our results are in contrast to most rodent studies. Detection of oMSC in head wounds and peripheral organs possibly indicating “competitive” homing signals. This phenomenon requires further investigation before clinical concept translation. (230 words)

POS-WED-170

FEMTOSECOND-LASER DENDROTOMY

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Purpose: Focused laser light has found use in cellular surgery as an ultra-sharp scalpel. The nonlinear nature of two-photon (2P) absorption localizes interaction to a narrow focus with minimal damage to surrounding tissue. We use a custom-built 2P laser microscope to prune the dendritic tree of layer V pyramidal neurons and assess how this affects firing. **Methods:** We imaged neurons filled with 100µM of Alexa-488 in 300µm slices of somatosensory cortex from 15-19 day-old rats using 12-22mW of 800nm laser light. The site of a potential cut was chosen from this image and cuts were made with 100ms pulses of 30-150mW at 720nm. The holding current of the neuron was monitored in whole-cell voltage-clamp and firing patterns in current-clamp were compared before and after the cut. Cuts were verified by generating EPSPs via 2P uncaging of MNI-glutamate at identified spines proximal and distal to the cut and via biocytin staining *post hoc*. **Results:** Dendritic cuts were followed by a sudden and large increase in holding current (>200 pA) and were evidenced by retained proximal uncaging responses but lost distal ones. They were verified in biocytin stainings proximal to the point of the cut. Cuts of small 3rd or 4th order dendritic segments did not change neuronal firing rate significantly. However, cuts of 1st and 2nd order segments resulted in an increase in firing rate for the same current injected. **Conclusion:** This approach can be used for arbitrarily pruning dendritic morphology and investigating the relationship between dendritic structure and neuronal function. (250 words).

POS-WED-171

FREQUENCY-SPECIFIC EFFECTS OF PMF STIMULATION ON NEURO-CIRCUIT REPAIR AND SINGLE CORTICAL CELLS IN VITRO.

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Electromagnetic fields are widely used to non-invasively stimulate the human brain in clinical treatment and research, applying either high-intensity fields (~1 Tesla, T) in repetitive transcranial magnetic stimulation (rTMS) or low intensity (µT-mT) pulsed magnetic fields (PMFs). While the effect of such stimulation varies according to frequency and intensity, mechanisms called into play by these parameters remain unknown.

We investigated the effects of different stimulation frequencies of low intensity PMF (12-14 mT) *in vitro*. We applied PMF to primary cortical cultures for 4 days and assessed survival and morphological changes. To understand underlying mechanisms, we measured intracellular calcium flux during PMF stimulation and changes in gene expression. We show frequency-specific effects of PMF stimulation: simple frequencies (1 Hz, 10 Hz and 100 Hz) impaired single cell survival, while more complex frequencies did not. Moreover, 1 Hz stimulation modified neuronal morphology, inhibiting neurite outgrowth. All frequencies induced calcium release from intracellular stores, with frequency-specific changes in gene expression related to apoptosis and neurite outgrowth.

We further investigated the effects of PMF on neuro-circuit repair in organotypic hindbrain cultures. Organotypic murine hindbrains were stimulated for 2 weeks after cerebellar denervation to assess axonal reinnervation and Purkinje cell survival. We show that stimulation with a complex frequency increases axonal reinnervation, while simple frequencies (1 Hz and 10 Hz) did not. No frequency affected Purkinje cell survival.

Our results highlight the biological importance of low intensity PMF stimulation either on its own or as a contributor to the effects of rTMS. (247 words).

POS-WED-172

MONITORING ALPHA-SYNUCLEIN FIBRILLISATION BY NOVEL AGGREGATION-INDUCED EMISSION FLUOROGENS

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α-Synuclein (α-Syn) filbrillisation whereby α-Syn monomers misfold and amalgamates into oligomers and finally amyloid fibrils, is the central mechanism in developing Lewy body pathology leading to Parkinson's disease and related synucleinopathies. Chemicals that detect α-Syn oligomers and fibrils are valuable diagnostic tools for synucleinopathies. Professor Tang's group in Hong Kong has recently discovered a novel class of fluorogens characterised by the aggregation-induced emission (AIE) property where emission is induced when the fluorogens bind ligands in higher concentrations, therefore overcome the intrinsic defect of aggregation caused quenching inherited by traditional fluorogens. We tested seven AIE fluorogens for their abilities of detecting distinguishing α-Syn monomers and firbils. Recombinant α-Syn of 100 μM was placed on an orbital thermomixer with constant agitation at 37ºC. α-Syn of 5 μM and 15 μM of AIE fluorogens were mixed and fluorescence was measured by a multimode plate reader at various time points. Thioflavain T (ThT, 15μM) α-Syn binding under the same conditions served as positive controls. Four out of the seven AIE fluorogens were capable of slectively binding α-Syn fibrils over monomers. Importantly, one of the fluorogens (TPE-TPP) can distinguish monomer, oligomer and fibril of α-Syn with high sensitivity and specificity. AIE fluorogens could be valuable tools in monitoring the kinetics of α-Syn fibrillation with high sensitivity and specificity, and some could distinguish between α-Syn monomer, oligomer and fibril thus may be useful for developing diagnotics for synucleinopathies.

POS-WED-173

IN VIVO MACROPHAGE POLARISATION INDUCED BY INTERLEUKIN 10 (IL-10) FUNCTIONALISED ELECTROSPUN NANOFIBRE SCAFFOLDS IN THE PERIPHERAL NERVOUS SYSTEM

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Macrophages are myeloid lineage cells that display plasticity in response to various environmental cues. In the peripheral nervous system (PNS), macrophages remove myelin ovoids formed during Wallerian degeneration and secrete cytokines and growth factors that support nerve regeneration. It has been suggested that macrophages shift from pro-inflammatory (M1 phenotype) to anti-inflammatory (M2 phenotype) states during wound repair in response to IL-4, IL-13 or IL-10. To overcome the short half-life of IL-10 *in vivo*, we stabilised the protein by covalently tethering it to electrospun poly-ε-caprolactone nanofibres. We then implanted functionalised scaffolds in the sciatic nerve of male Wistar rats. Sciatic nerves were collected 1d (n=5), 3d (n=5), 7d (n=5) and 14d (n=5) after implantation to quantify M2 macrophage sub-populations. IL-10 was quantified on the scaffold's surface using enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry was used to quantify macrophage sub-populations. We demonstrate that IL-10 remains attached to the scaffold surface for up to 4 months at room temperature in PBS. There was no significant difference in the density of activated macrophages (ED1+) in the nanofibre region between control and IL10-treated groups, however cell density was higher inside the underlying nerve in the IL10-treated group ($p=0.05$). This group also showed a significant increase in the ratio of M2 cells (Arginase1+ED1+ / ED1+) in the nanofibre region at days 1 ($p<0.0004$), 3 ($p<0.0051$), 7 ($p<0.0007$) and 14 ($p<0.006$), and inside the nerve region at days 1 ($p<0.0001$) and 3 ($p<0.04$). Ability to manipulate macrophage phenotype may prove useful for treating nervous injuries.(Word count = 248)

POS-WED-174

OPERANT CONDITIONING BOXES TO INVESTIGATE THE EFFECTS OF LPS INDUCED ANHEDONIA; PROVIDING THE LINK BETWEEN NEUROINFLAMMATION AND BEHAVIOURAL RESPONSES

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How do you know you feel sick? When suffering from an illness the body must react to protect itself. Infection results in activation of the innate immune system triggering immune-to-brain communication through actions of proinflammatory cytokines altering behaviour through the “sickness response”. A prominent change with sickness is anhedonia, the reduced pleasure from rewarding stimuli. Current preclinical rodent models utilise behavioural tests such as saccharine two-bottle choice. However, such measures only provide information concerning the total response over the access period and do not inform about changed behaviour during tis time. To investigate this further we unified the saccharin two-bottle choice test (2 h access to 0, 1, 10 30 or 30 mM), with the precision of operant conditioning chambers, to gain greater insight on patterns of anhedonic behaviour. Additionally, inter-strain differences in anhedonic response to immune challenge will be quantified in Balb/c, CBA and C57Bl6 strains (n=6/group). Preliminary data from Balb/c mice demonstrate significant advantages of quantifying behaviour over time compared to solely endpoint preference analysis, given the near perfect saccharin concentration response (R^2=1.0) when analysing preference area under the time curve compared to only the total of saccharin lick preference (R^2=0.8). Ongoing studies will examine the impact of immune challenge (i.p. endotoxin) has on this behaviour and the inter- strain differences in illness anhedonic response.

Word Count: 216

POS-WED-175

TRPV1-IMMUNOREACTIVE NERVE TERMINALS IN THE CORNEAL EPITHELIUM.

Alamri AS, Ivanusic JJ, Brock JA.

A large proportion of sensory nerve terminals innervating the corneal epithelium are polymodal nociceptors that are activated by noxious thermal, chemical and/or mechanical stimuli. TRPV1 is a key ion channel that is thought to be involved in nociception, and is often used as a marker of polymodal nociceptors. The aim of this study was to determine if there are morphologically and/or neurochemically distinct subpopulations of TRPV1-immunoreactive nociceptors innervating the corneal epithelium. ***Methods:*** We used double label immunohistochemistry and high-resolution confocal microscopy to explore the morphology and neurochemistry of nerve terminals in whole mounts of guinea pig and mouse cornea. ***Results:*** In both species (*n=7* GP; *n=5* mice), TRPV1-like immunoreactive (-LI) axons arose directly from leash fiber bundles and projected up towards the surface of the corneal epithelium where they terminated in the wing cell layer a few micrometers from the surface in a single simple bulbar ending, or at the surface of the epithelium with three to four horizontally ramifying fibres each with a simple ending. In the guinea pig cornea, double labelling revealed that terminals with simple morphology were CGRP-LI but not GFRα3-LI (*n=3*), and TRPV1-LI terminals with ramifying morphology were GFRα3-LI but not CGRP-LI (*n=2*). By contrast, in the mouse cornea, double labelling revealed that both simple and ramifying TRPV1-LI nerve terminals were CGRP-LI and GFRα3-LI (*n=1*). ***Conclusion:*** This study indicates that there are morphologically and neurochemically distinct sub-populations of corneal polymodal nociceptors in the guinea pig, and that this feature may be species specific. (247 words).

POS-WED-176

A BIOLOGICALLY INSPIRED FACILITATION MECHANISM ENHANCES THE DETECTION AND PURSUIT OF TARGETS OF VARYING CONTRAST

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Detecting and robustly tracking a small moving object against a cluttered background is a challenging task for both natural and artificial visual systems. Within this context, insects are an ideal group to draw inspiration with a light-weight and low-power neuronal architecture underlying their low-resolution visual system (~1°). Many species of flying insects detect and chase prey or conspecifics within a visually cluttered surround, e.g. for predation, territorial or mating behavior. We modeled such detection and pursuit for small moving targets, and tested it within a closed-loop, virtual reality flight arena. Our model is inspired directly by electrophysiological recordings from 'small target motion detector' (STMD) neurons in the insect brain that are likely to underlie this behavioral task. The front-end uses a variant of a biologically inspired 'elementary' small target motion detector (ESTMD), elaborated to detect targets in natural scenes of both contrast polarities (i.e. both dark and light targets). We also include an additional model for the recently identified physiological 'facilitation' mechanism believed to form the basis for selective attention in insect STMDs, and quantify the improvement this provides for pursuit success and target discriminability over a range of target contrasts. We ran this model in simulation of 600 random pursuit paths of a fly-sized target of variable brightness, viewed against 3 different background images. Facilitation improved pursuit success for low-contrast features by up to 35% in the best-case scenario (Chi square=7.55, p=0.006). (250 words).

POS-WED-177

SENSORY NERVE FIBRES CONTAINING CALCITONIN GENE-RELATED PEPTIDE IN BACK MUSCLES AND FASCIAE.

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Little is known about the sensory innervation of back muscles and their associated fasciae. Nociceptors containing substance P (SP) and calcitonin gene-related peptide (CGRP) and expressing TRPV1 channels mediate pain following tissue damage and inflammation. We aimed to identify the distribution of nerves expressing these markers in back muscles and fasciae of mice.

Methods

The thoracolumbar fascia, latissimus dorsi, erector spinae and multifidus muscles were sampled from C57Bl/6 mice (n = 12). Nerve fibres were identified by immunoreactivity (IR) to neuron specific enolase (NSE), and subpopulations of single varicose fibres expressing immunoreactivity to SP, CGRP and TRPV1 were mapped.

Results

Most free varicose nerve fibres with NSE-IR in muscle and in the thoracolumbar fascia contained CGRP-IR. About half also contained SP-IR and this proportion was similar in muscles and fascia. TRPV1-IR was expressed by fibres containing both peptides but not by those expressing CGRP-IR without SP-IR. Fibrous and fatty areas of the thoracolumbar fascia contained numerous CGRP-IR and SP-IR fibres. Compared to muscles, the fascia was more densely innervated. In latissimus dorsi, the number of fibres was highest near the fascial attachments. In comparison, the intrinsic muscles of the back contained relatively few single nerve fibres.

Conclusions

Although the thoracolumbar fascia and muscles of the back contain peptidergic sensory nerve fibres that may contribute to pain and inflammation, a high proportion of afferent fibres contain CGRP but not substance P. The role of these nerves in the development of back pain remains unknown. (word count 243)

POS-WED-178

GREEN EYES - CANNABINOID MODULATION OF VISUAL FUNCTION IN MICE

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Our understanding of a visual cannabinoid system has grown in the past decade. Endocannabinoids have been ubiquitously found throughout the nervous system. Retinal cells express cannabinoid receptors (CBr) that are capable of altering voltage-dependent currents via reduction in glutamate and GABA release. The foundation studies of CBr receptor activity has been limited to single and whole cell recording. We tested the effect of CBr partial agonist *in vivo* using the electroretinogram (ERG) in rodents.

Compared to time controls systemic injection of WIN-55212-2 a non-specific cannabinoid agonist increased the photoreceptor and inner retina derived ERG. The normalised full field ERG from dim to bright photoreceptor a-wave was increased compared time controls); dim (-9% win injected vs. -31% sham, $p < 0.05$), mid (-23% vs. -32%, $p < 0.05$) and bright (-5% vs. -29%, $p < 0.05$). The inner retina b-wave changes were dependent on light intensity; dim (-19% win injected vs. -23% sham, $p = 0.12$), mid (7% vs. -17% sham, $p < 0.05$), bright (-8% vs. 23%, $p < 0.05$). Non-specific cannabinoid receptor agonism appears to modulating both photoreceptor and bipolar, horizontal and amacrine conductances *in vivo*, consistent with whole cell recordings.

Our results expand our current knowledge of retinal circuitry, and builds on earlier studies. The implication of cannabinoid modulation in the retina support further studies on the effects of cannabis use on vision, and unintended side-effects of cannabinoid based drugs in glaucoma treatments. (233)

POS-WED-179

α-CONOTOXIN VC1.1 TARGETS $GABA_B$ RECEPTORS MODULATING N-TYPE ($CA_V2.2$) CALCIUM CHANNELS IN SENSORY PATHWAYS MEDIATING CHRONIC VISCERAL PAIN

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Introduction: α-Conotoxin Vc1.1 is a small disulfide-bonded peptide isolated from the venom of *Conus victoriae*. Although Vc1.1 has been demonstrated to have anti-nociceptive actions in animal models of neuropathic pain its applicability to chronic visceral pain is currently unknown.

Methods: Using *in vitro* electrophysiological recordings we determined the effects of Vc1.1 (1-1000nM) on colonic nociceptors in healthy mice and those with chronic visceral hypersensitivity (CVH). We assessed the ability of Vc1.1 to alter the transmission of nociceptive signaling *in vivo* by measuring the activation of dorsal horn neurons in the thoracolumbar (T10-L1) spinal cord in response to noxious colorectal distention. mRNA and protein expression of $GABA_B$ receptor subunits and $Ca_V2.2$ were determined in retrogradely traced colonic DRG neurons using quantitative RT-PCR and immunohistochemistry, respectively.

Results: The $GABA_B$ receptor, a target of Vc1.1, and its down-stream effector channel $Ca_V2.2$ were highly expressed in colonic DRG neurons from both healthy and CVH mice. In healthy mice, Vc1.1 caused dose-dependent inhibition of colonic nociceptor mechanosensitivity (P<0.001,n=10). This inhibitory effect of Vc1.1 was significantly greater in CVH nociceptors (P<0.001,n=11). Notably, the Vc1.1-induced inhibition of colonic nociceptors was reduced by the $GABA_B$ receptor antagonist CGP55845 (5μM;n=6-9) and by the selective $Ca_V2.2$ inhibitor ω-conotoxin CVID (1μM;n=8-9). *In vivo* intra-colonic administration of Vc1.1 significantly reduced signaling of noxious colorectal distention to the spinal cord (P<0.01,n=4). This effect was also significantly greater in CVH mice (P<0.001,n=4).

Conclusions: The enhanced anti-nociceptive actions of Vc1.1 during CVH suggest it is a novel candidate for the treatment for chronic visceral pain.

(250 words)

POS-WED-180

ACTIVATION OF EXTRINSIC SENSORY NEURONS ASSOCIATED WITH MOTILITY OF THE ISOLATED GUINEA PIG COLON

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Extrinsic primary afferent neurons mediate reflexes and sensations from the gastrointestinal tract. Capsaicin-sensitive spinal afferents, with endings on intramural and extramural blood vessels, are a major mechano-nociceptive pathway. These sensory neurons can be activated by chemical stimuli (including inflammatory mediators) and by experimental distension. However, sensory neurons are also activated, in vivo, during movements of the gut, but the effective patterns of activity have not been clarified. We combined high resolution intraluminal fibre-optic manometry (1cm sensor spacing with a 1.3mm diameter catheter) with spatiotemporal diameter mapping and extracellular recording of sensory neuron firing from colonic nerves innervating 4cm long specimens of guinea pig colon, *in vitro*. Sensory firing could be related specifically to mechanical changes (pressure, diameter) in the sensory neuron's field of innervation, with high spatial and temporal resolution. Low levels of spontaneous firing increased dramatically during luminal distension with Krebs solution to 4, 7.5, 15, 30mmHg (16±13/min control, 204±136/min 15mmHg, n=6 $P<0.01$). Bursts of sensory firing correlated strongly with waves of contraction evoked by distension. Addition of 1uM nicardipine with 1uM hyoscine led to abolition of distension-evoked phasic contractile activity, leaving passive responses to distension. Sensory responses were reduced, lacked bursts and showed slowly adapting firing. Most of the responsive afferents were activated by bath application of 1uM capsaicin, suggesting that they belonged to the putative class of mechano-nociceptors. The data indicate that contractile activity of the muscularis externa, rather than distension, is a major determinant of the activation of extrinsic sensory neurons to the gut. (248 words).

POS-WED-181

SPINAL CORD PAIN PROCESSING – ABSENCE OF SPHINGOSINE KINASE 2

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Purpose & Methods: Although sphingosine 1-phosphate (S1P) signalling is involved in chronic inflammation, the impact of Sphk2-generated S1P in acute inflammatory pain is not clear. We compared the nocifensive behaviour and spinal cord transcription factor activation in response to formalin-induced acute inflammation in C57BL/6 wild-type and sphingosine kinase 2 deficient ($Sphk2^{-/-}$) mice. Immunoreactivity for pCREB and c-fos was detected in the dorsal horn. GFAP, Iba-1 and NeuN were used to label astrocytes, microglia and neurons, DAPI was used as a nuclear stain. Positive labelling was analysed with Image J.

Results: Compared with C57BL/6 wild-type (wt) mice, $Sphk2^{-/-}$ mice showed significantly reduced levels of S1P but similar activity of the Sphk1-isoform in the spinal cord (n = 5). Analysis of nocifensive licking behaviour in response to different concentrations of formalin showed (a) a greater 1^{st} phase response in female compared to male mice (n = 3-9/group) and (b) an earlier 2^{nd} phase response with increased licking times in male $Sphk2^{-/-}$ mice compared to wt (n = 5). Analysis of pCREB and c-fos expression 60 min after formalin injection showed bilateral increase in labelling with higher c-fos numbers in females (n = 3-9/group). **Conclusion**: Sphk2 is the predominant Sphk isoform in the spinal cord. Absence of this isoform changes the 2^{nd} phase response to acute inflammation in male mice indicating modulation of central sensitization. Deficiency in Sphk2 also changed the activation pattern of transcription factors in ipsi-and contralateral spinal cord. This suggests a Sphk2-generated S1P modulates bilateral spinal cord pain processing.

POS-WED-182

DIFFUSE TRAUMATIC BRAIN INJURY AND CORTICAL INHIBITION

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Traumatic brain injury (TBI) produces several changes at the cellular level such as axonal and dendritic injury, astrocytosis and excitation-inhibition imbalance. We have previously shown that a long-term effect of diffuse TBI is hyper-excitation in supragranular sensory cortex. Such increase in cortical excitation following TBI is thought to be mainly due to loss or reduced activity of inhibitory neurons but this has not been directly examined. Hence, we investigated the TBI-induced changes in the numbers and distribution of different subtypes of cortical inhibitory neurons, characterising the changes in the expression of two calcium binding proteins, calbindin and parvalbumin, 8 weeks post TBI. TBI was induced using the weight drop impact acceleration (WDIA) method and sham controls received surgery only. Rats were euthanized 8 weeks post TBI, and brains perfusion fixed and wax embedded. Immunohistochemistry on 10 µm sections was performed to identify calbindin and parvalbumin immunopositive cells in TBI and sham animals. There was a decrease in calbindin immunoreactive (CB-IR) neurons in deep layer III and layer IV and an increase in layer V ($p<0.05$). No statistically significant difference in the amount of parvalbumin immunoreactive neurons was seen in layers I-V. These findings reflect dynamic activity as a consequence of TBI-induced cortical reorganisation in inhibitory circuits. Long term hyperexcitability in diffuse TBI is therefore more likely a result of reduction rather than a loss of inhibition.

POS-WED-183

MICROSACCADE-LIKE RETINAL MOVEMENTS IN THE BLOWFLY *CALLIPHORA STYGIA*

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For several decades, electrophysiological recordings from the insect visual system have helped elucidate principles and mechanisms underlying 'elementary motion detection'. Such analyses consider that large-scale head and body movements contribute to the motion experienced. However, the contribution of smaller eye movements has largely been ignored. Franceschini and Chagneux (1997, 2001) proposed that flies change their gaze over a small scale (similar to vertebrate microsaccades) via coordinated activity of two intraocular muscles attached to the back of the retina. Distortion of the retina induced by contraction of these muscles can potentially shift the visual axes of photoreceptors behind the fixed dioptrics. To test this, we quantified retinal movements in the blowfly (*Calliphora stygia*) by intracellular recording from photoreceptors *in vivo*, whilst presenting a rapidly scanning feature. Variation in the time-to-peak of the response indicates the change in location of the receptive field centre. We show a significant increase (* $P<0.05$) in the variance of the receptive field location, consistent with retinal movements of approximately ±0.5°. As with vertebrate microsaccades, the function of these movements is not clear since they were not modulated by local and wide field visual stimulation (e.g. progressive and regressive gratings, moving features). Much modelling of this otherwise well-studied motion detection system assumes stationary visual input. Future research thus needs to take account of the dynamic variation in input due to retinal movements, which may have important consequences for downstream visual processing. (234 words).

POS-WED-184

NON-INFORMATIVE VISION ENHANCES TACTILE ACUITY: A SYSTEMATIC REVIEW.

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This systematic review aimed to compile and evaluate the available evidence as to whether non-informative vision of the body has an effect on tactile acuity. Vision was defined as non-informative in that there was no visual information about the tactile stimulus given. Studies that assessed tactile acuity with vision of the body, as compared to vision of a neutral object or vision occluded, were systematically identified and reviewed using a standard PRISMA protocol.

All of the nine studies included in this review were randomised, within subject, controlled trials published in English. Despite the diversity of protocols and outcome measures used, eight out of the nine studies reported improvements in tactile acuity when vision of the relevant body part was available. There were statistically significant findings from grating orientation demonstrating a significant positive effect of vision of the body, but statistically high heterogeneity (p = 0.002, *SMD* 3.31, 95% *CI* 1.24, 5.39, I^2 91%).

This systematic review demonstrates that non-informative vision of the body does influence and enhance tactile acuity. The conventional clinical approaches to tactile sensory training have stipulated that vision is occluded during training. However, the results from this review raise the question of whether this may be sub-optimal practice.

Based on the results of this systematic review, it may be necessary to review the conventional approach to vision during tactile sensory training and further research into the multisensory integration of touch and vision is recommended. (237 words)

POS-WED-185

NEURAL ADAPTATION OF THE N170 IS AFFECTED BY ADAPTOR STIMULUS DURATION AND INTERSTIMULUS INTERVAL.

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Event-related potential (ERP) neural adaptation studies of the N170 have been used to delineate the time course of neural processes in face and object perception. However, study designs have varied widely in regards to the duration of the adaptor stimulus and the interstimulus interval (ISI), and the combined effects of these have not been systematically tested. We measured ERPs to images of faces and chairs (representing nonface objects) in a Double Pulse paradigm in which an adaptor stimulus was followed by an ISI and then a test stimulus. Adaptor stimulus duration was varied between 200ms, 500ms and 1000ms and ISI was set at 200ms and 500ms duration. N170 peak amplitude measures were derived from 64-channel EEG recordings. Test stimuli-evoked N170s were smaller (more positive) following longer adaptor stimulus durations and longer (500ms) ISIs. Face and chair test N170 amplitudes differed by adaptor duration only when adapted by the same stimuli category, although differences by adaptor duration were found in faces but not chairs using 500ms ISIs. These results demonstrate that N170 peak amplitude is affected by adaptor duration and ISI, and that these factors may explain differences in the extent of neural adaptation in previous studies of high-level vision. Differences across adaptor duration appear to be category selective; that is that face and chair adaptors have different adapting effects on face and chair test stimuli. (226 words).

POS-WED-186

BEHAVIOURAL INDICATORS OF PERCEPTUAL STATE

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Aim. Perception is easier to experience that to measure. When a subject views an ambiguous stimulus, for example, the percept varies between two alternatives and the current percept is determined by asking the subject. This is problematic because the subject's criterion is typically variable. Our aim was to see whether a subject's current percept could be determined more objectively from non-verbal behavioural indicators. **Method.** Binocular rivalry, the alternating percept resulting from incompatible monocular stimuli, was induced by presenting an oblique grating to one eye and an orthogonal grating to the other eye. A brief test stimulus was added to one eye's rivalry stimulus at random times and contrasts. The test was presented at one of two spatial locations and the subject indicated which alternative had been shown. Response correctness and reaction time were recorded. The same quantities were also measured using binocularly congruent (and therefore non-rivalling) stimuli. **Results.** Given the random timing of the test stimulus, it was sometimes delivered when the tested eye was dominant and, at other times, suppressed. Accordingly, the psychometric function recorded during rivalry should be a mixture of the dominance and suppression forms of the function. As expected, the psychometric function during rivalry fell below that obtained with a binocularly congruent stimulus. Reaction times during rivalry were on average higher than those during congruent viewing, but the overlap between the two states was substantial. **Conclusions.** By pooling over many trials, response correctness and reaction time can both be used to indicate perceptual state.

[248]

POS-WED-187

DISTRIBUTION OF VISUAL AND SOMATOSENSORY SIGNALS IN SUPERIOR COLLICULUS OF RAT

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The superior colliculus (SC) is known to be important in orienting behaviour. In rodents SC receives visual, auditory and somatosensory inputs, but little is known of whether and how neurons combine information from different sensory modalities. Here, we compare responses of neurons in SC of rat to visual and somatosensory (whisker) inputs. Extracellular recordings were made from SC of isoflurane-anaesthetised Long-Evans rats. Visual stimulation was an increment or decrement in light from a grey background, at each of 5 contrast levels. Somatosensory stimulation was a vibrating mesh that contacted all whiskers, at each of 5 vibration amplitudes. We used multi-electrode arrays to map visual- and whisker-related multi-unit activity, with measurements from 168 electrode contacts at 16 recording sites (4 animals). Visually responsive electrodes (n = 94) were in superficial and intermediate layers. Whisker responsive electrodes (n = 54) were in the intermediate layers. Response to both visual and whisker stimulation was observed at 20 electrodes in the intermediate layers. To determine if individual neurons integrated signals from the two modalities we made additional single-unit measurements from 61 neurons (7 animals). In each case response was well described by a sigmoidal function in the joint space of visual and whisker stimulation. Single-units were visually responsive (n = 49) or whisker responsive (n = 12); 2 neurons showed significant response to both visual and whisker stimulation. Our observations suggest that there is overlap in the representations of whisker and visual signals in SC of rat, but with little integration in individual neurons. (250).

POS-WED-188

COMPARISON OF TECHNIQUES FOR AUTOMATED MAPPING OF RECEPTIVE FIELDS OF LARGE NEURONAL POPULATIONS IN PRIMATE CORTEX

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Purpose: A comprehensive understanding of visual cortex requires data obtained using electrophysiological recordings in animal models. There is a need for methods that allow combination of extensive, regular sampling of a given area (or border between areas) with quantitative methods. Here, we report on the use of multi-electrode arrays to advance this issue.

Methods: Six marmosets (Callithrix jacchus) were anaesthetized with sufentanil (8 µg/kg/h) and N2O (70% in oxygen), and the eyes were focused on a computer screen used to display visual stimuli. Recordings were obtained through 96-channel “Utah” arrays, inserted in visual areas including V1, V2, DM, and MT. We compared the advantages of three automated methods in term of receptive field accuracy and data collection time.

Results: The spatial attributes of receptive fields were similar across the three methods, but temporal information was best obtained using reverse correlation. Stimulation with long bars moved in different directions required only minutes to characterise the spatial receptive field, but yielded no temporal information and was highly sensitive to estimations of response latencies. White noise reverse correlation accurately characterised spatial and temporal properties of the receptive fields but required hours of data collection for optimal results. Sparse correlation using single flashed stimuli provided accurate spatial maps with good signal-to-noise ratio, but required a shorter acquisition time than white noise reverse correlation.

Conclusion: Automated routines will allow an unprecedented level of quantitative insight on sensory processing by neuronal populations. The choice of the appropriate algorithm highly depends on the specific needs of applications. [250 words]

POS-WED-189

SPEED PERCEPTION FOLLOWING ADAPTATION IS NOT INFLUENCED BY GLOBAL ACCELERATION PATTERNS

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Adaptation to a stimulus of a certain speed often results in a reduction of perceived speed and an increase in speed discriminability and in certain circumstances can result in an increase in perceived speed. A total of 6 naïve subjects participated in the study. In this experiment I presented expanding dot flow fields with accelerating (global), decelerating (global) and mixed accelerating/decelerating (local) speed patterns in an attempt to determine the relative contribution of local versus global velocity on the perceived speed of the stimulus following adaptation. I show that the greatest decreases in perceived speed are found when viewing low test speeds after adaptation to high speeds, and small increases in perceived speed occur when viewing high test speeds after adapting to low speeds. There are small but significant differences in perceived stimulus speed after adaptation due to different acceleration profiles. These effects, while significant, accounted for less than 2% of the total variation in the perceived speed and speed discriminability of the stimuli. As such the speed perception of expanding stimuli following adaptation is predominantly influenced by the speed of the local components. The global relationship between the speeds of local elements has little effect on the perceived speed or discriminability of the stimulus.

(205 Words)

POS-WED-190

STRETCH AND DISTENSION OF MESENTERIC BLOOD VESSELS INCREASES EXTRINSIC AFFERENT DISCHARGE IN THE ISOLATED GUINEA PIG ILEUM.

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Afferent nerves with activation sites located near blood vessels in both mesenteric membranes and within the gut wall have been previously identified as a major mechano-nociceptive pathway (Song et al 2009) and are referred to as "vascular afferents". In this study, *in vitro* extracellular recordings were made from mesenteric nerves innervating 2cm long specimens of guinea pig ileum. Nerves were crushed before entering the gut so that only mesenteric inputs were recorded, but vascular continuity with the gut was preserved. Preparations were set up so that the mesenteric blood vessels could be stretched longitudinally via a counter-weight or distended via a cannula in the artery connected to a reservoir of modified Krebs solution that could be raised to a specific height (up to ~60mmHg). Both stretch and distension of the mesenteric blood vessels resulted in an increase in firing rate (0.045±0.024Hz control, 1.02±0.38Hz 15g stretch, n=18, $P<0.05$ and 0.054±0.018Hz control, 0.43±0.11Hz 60mmHg distension, n=34, $P<0.01$). Distension-induced firing adapted quickly but did not return to basal firing rate. We tested whether stretch of the mesentery during maintained distension of the vessels had a greater effect on change in firing rate compared to stretch alone. The results showed no significant difference in stretch responses in distended versus non-distended mesenteric vessels. Overall, the data suggests distension within an approximately physiological range can excite vascular afferents, as can mesenteric traction. However, the combined effect of these two mechanical stimuli does not appear to be additive. (241 words).

Song XY et al (2009) Gastroenterology 137 (1) 274-284

POS-WED-191

SELECTIVE INSECT TARGET TRACKING USING A BIOPHYSICALLY INSPIRED SPIKING WINNER-TAKE-ALL NETWORK.

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Dragonflies are extraordinary insects that possess fine-tuned neural pathways for efficiently computing complex visual tasks in real-time. Such capabilities include; optic flow analysis, collision avoidance and target tracking in visually cluttered environments, while avoiding natural predators. Past studies have investigated the neural circuitry and computations that underlie such behaviour. An advantage over their mammalian counterparts, is that the insect visual system has greatly reduced structural complexity, without compromising the capacity to solve complex visual tasks. Using electrophysiological techniques, we investigate the neuronal architecture that permits dragonflies to detect and track very low contrast moving targets against complex continuously changing background clutter, as well as to direct attention to the primary target amidst the distracting motion of nearby objects. Recent experiments have demonstrated that small target motion detector neuron (CSTMD1) uses an attention mechanism that competitively selects one moving target from alternatives.

Here, we present a biophysically plausible spiking neural network model of the 'small target motion detector' pathway. This model includes a 2D array integrating size-selective inputs, which in turn stimulate a global integrating neuron (CSTMD1). The network uses competitive selection where spiking neurons stimulated by AMPA and NMDA synaptic inputs along with inhibition globally feeding back from the integrative unit to the array of STMD neurons. Our model displays a winner-take-all behaviour mimicking selective attention akin to those seen in recent experiments involving individual dragonfly STMD neurons. Finally, we illustrate how the balance between excitation and inhibition impacts selective attention. Significantly, our results provide biophysical insights to the origins of selectively attending to single targets.

POS-WED-192

PIEZO2 EXPRESSION IN CORNEAL AFFERENT NEURONS

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The molecular identities of channels that confer noxious mechanical sensitivity to peripheral sensory neurons remain largely elusive. Recently, a novel class of mechanically sensitive channels has been identified and have been called Piezo channels. In this study we explored Piezo channel expression in sensory neurons supplying the guinea pig corneal epithelium, which have well defined modalities in this species. We hypothesized that a proportion of corneal afferent neurons express Piezo2, and that these neurons are neurochemically distinct from corneal polymodal nociceptors and cold-sensing neurons, which contain calcitonin gene-related peptide (CGRP) and the cold sensing ion channel TRPM8, respectively. We used a combination of retrograde tracing to identify corneal afferent neurons and double label *in situ* hybridization and/or immunohistochemistry to determine their molecular and/or neurochemical profile. We found that Piezo2 expression occurs in approximately 30% of corneal afferent neurons. Piezo2 corneal afferent neurons are almost exclusively medium to large sized neurons that are NF200-immunoreactive (IR) but are not CGRP-IR. This suggests they are not corneal polymodal nociceptors. Using double *in situ* hybridization there was no co-expression of Piezo2 and TRPM8 transcripts in any trigeminal ganglion neurons, further suggesting that Piezo2 is not expressed in corneal cold-sensing neurons. Thus Piezo2 expression occurs in a neurochemically distinct sub-population of corneal afferent neurons that are not polymodal nociceptors or cold-sensing neurons, and is likely confined to a sub-population of pure mechano-nociceptors in the cornea. This provides evidence that Piezo2 is a strong candidate for a channel that transduces noxious mechanical stimuli.

(word count = 247)

POS-WED-193

PREDOMINANCE OF RADIAL ORIENTATIONS IN THE ORIENTATION MAP OF THE MACAQUE PRIMARY VISUAL CORTEX

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Introduction: Most neurons of the primary visual cortex (V1) are orientation selective. Origin of this selectivity is still a matter of debate. One hypothesis is that orientation selectivity in the cortex arises from orientation anisotropies that may be present even at the level of retina. These anisotropies are mostly biased for the radial orientation, i.e., orientation of the line joining the receptive field centre to fovea. In this study, we tested the hypothesis that cortical orientation selectivity is built upon the orientation selectivity seen subcortically and hence the distribution of preferred orientations in the cortex will also show a similar radial bias.

Methods:. We imaged the neuronal response of V1 in 2 macaques using optical imaging of intrinsic signals. We then found the preferred orientation of the signals within a moving region of interest (800 µm x 800µm stepped every 400µm) by calculating the circular mean. Electrophysiological recordings revealed the receptive fields of defined locations in the imaged area. The preferred orientation obtained from optical imaging was compared with the radial angle corresponding to that location.

Results: We found that the radial orientation was over represented in the imaged area of V1 (Chi square= 289.59, df=17, $p<0.001$). Average difference between the radial angle and computed optimum orientation of the imaged intrinsic signal was 18.8 ± 1.8 degrees (±SEM).

Conclusions: Our results indicate that there is an over representation of the radial orientations in the afferent and synaptic activity in macaque V1, possibly reflecting the radial pattern seen in retinal responses.

POS-WED-194

NEURAL BASIS OF BINOCULAR RIVALRY IN PRIMARY VISUAL CORTEX OF THE MOUSE

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Binocular rivalry is a form of visual competition that occurs when the eyes view conflicting images simultaneously. Although cortical inhibition in early visual areas has been suggested to account for binocular rivalry, the underlying synaptic mechanisms are unknown. Here we investigated the response of binocular neurons in primary visual cortex of the mouse to dichoptic incongruent visual stimuli known to trigger binocular rivalry in humans and primates. *In vivo* whole-cell recordings were made from simple cells in layer 2/3 of anaesthetized adult mice. Sinusoidal drifting gratings were presented to each eye alone or to both eyes together with the same or orthogonal orientation and changes in AP firing and subthreshold membrane potential determined as the ratio of the binocular response to the contralateral response alone. As expected, presentation of binocular stimuli with the same orientation increased AP firing relative to contralateral stimulation (binocular/contralateral ratio: 3.2 ± 0.8, n=9). In contrast, binocular stimuli with orthogonal orientation led to a decrease in AP firing (binocular/contralateral ratio: 0.6 ± 0.2; n=9; p=0.009). Subthreshold changes in membrane potential were also reduced during presentation of binocular stimuli with orthogonal orientation, although this effect was smaller and not statistically significant (binocular/contralateral ratio: 1.3 ± 0.1 for binocular gratings with the same orientation compared to 1.0 ± 0.1 for orthogonal binocular gratings; n=13; p=0.1). These findings suggest that viewing of binocular conflicting images leads to either enhanced inhibition or decreased excitation to cortical neurons. These changes may play a role in binocular rivalry. (246 words).

POS-WED-195

NOCICEPTIVE DORSAL HORN NEURONS RECEIVE CONVERGENT CONTACTS FROM PRIMARY AFFERENT TERMINALS EXPRESSING CALCITONIN GENE-RELATED PEPTIDE BUT NOT SUBSTANCE P

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Purpose: Calcitonin gene-related peptide (CGRP) immunoreactivity (IR) is commonly used to identify peptidergic nociceptors. However, many CGRP-IR neurons lack another nociceptive marker, substance P (SP). The targets of these neurons are unknown. Therefore, using the phosphorylated extracellular signal-regulated kinase (pERK) and cAMP response element-binding protein (pCREB) as markers for neuronal activation, we stimulated inputs to spinal cord slices *in vitro* and used multiple labelling immunohistochemistry to determine whether spinal nociceptive neurons received contacts from terminals containing CGRP but not SP.

Methods: Spinal cords were removed from C57Bl/6 mice and sliced on a Vibratome at 500 µm. Slices were incubated at 37°C for 3 h before either electrical stimulation of dorsal roots or chemical stimulation with 10^{-6}M capsaicin. Slices were re-sectioned at 20 µm and pERK-IR or pCREB-IR was detected on sections also labelled for IR to CGRP and SP.

Results: CGRP terminals lacking SP were most prominent in lateral areas of lamina I and in lamina IV (n=4 animals). After both electrical (n=4) and capsaicin stimulation (n=8), numbers of cells labelled for pERK/pCREB increased significantly compared to controls in lamina I, but not lamina IV/V. In lamina I, CGRP terminals lacking SP made contacts with pERK/pCREB positive cells that also received contacts from CGRP terminals containing SP (n=8).

Conclusions: CGRP neurons lacking SP have dual central termination sites in the spinal dorsal horn. They may modulate nociceptive input in lamina I, but not in lamina IV/V. These neurons are not likely to be involved in acute nociception. (247 words).

POS-WED-196

TUBB5 AND ITS DISEASE-ASSOCIATED MUTATIONS INFLUENCE THE TERMINAL DIFFERENTIATION OF CEREBRAL CORTICAL NEURONS

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The microtubule cytoskeleton is critical for the generation and maturation of neurons in the developing mammalian nervous system. We have recently reported that the tubulin isoform, Tubb5, mediates cerebral cortical development, and that missense mutations detected in human subjects were pathogenic for neurodevelopment *in vivo* (Breuss et al, Cell Reports, 2012). In this study, we focused on the role for Tubb5 in neurodifferentiation and report that changes to its expression impair the maturation of newborn neurons within the developing mouse cerebral cortex. We find that Tubb5 knockdown leads to alterations in their differentiation, and forced expression of the pathogenic variants of TUBB5 differentially impair the terminal differentiation of cortical neurons. Therefore, our observations highlight the importance of *Tubb5* as a critical gene for neurodifferentiation, and provides insight into the underlying cellular pathology associated with TUBB5 disease states.