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ORAL-01-01

SPONTANEOUS Ca^{2+} WAVES PROPAGATE BETWEEN ADJACENT NEURAL CREST-DERIVED CELLS DURING ENTERIC NERVOUS SYSTEM DEVELOPMENT BUT ARE NOT MEDIATED BY GAP JUNCTIONS

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Aim: Propagating waves of activity are seen in many parts of the developing nervous system and are important for the formation and maintenance of connections between neurons. The enteric nervous system (ENS) arises from enteric neural crest-derived cells (ENCCs) that migrate into the developing gastrointestinal tract and differentiate to form neurons and glia. Although there is abundant evidence that ENCCs act as a population, and therefore must communicate with each other, little is known about how ENCCs communicate. In this study we examined spontaneous changes in intracellular Ca^{2+} in ENCCs in the intact embryonic gut during ENS development. Methods: These changes were examined using *Wnt1-CRE;R26R-GCaMP3* mice, in which all neural crest-derived cells express the genetically-encoded Ca^{2+} indicator, GCaMP3. Isolated segments of intact embryonic gut were imaged at 1 Hz. Results: Spontaneous Ca^{2+} transients lasting several seconds were recorded from some individual ENCCs. Spontaneous propagating Ca^{2+} waves were also seen, where Ca^{2+} transients in one ENCC spread to adjacent ENCCs. Propagating Ca^{2+} waves were most frequently observed at E11.5-E13.5, at approximately 8 per hour. On average, 7 ENCCs were involved in each wave. The gap junction inhibitors, carbenoxolone (n=5) and glycyrrhetinic acid (n=6), had no effect on the frequency and propagation of Ca^{2+} waves. Wave-like activity could also be elicited using focal electrode stimulation (30V, 400µsec). Conclusions: This is the first direct identification of communication between ENCCs during ENS development. The propagation of waves does not require gap junctions; we are now investigating other possible mechanisms. (Word count = 245)

ORAL-01-02

SPINAL AFFERENTS STIMULATE MAST CELLS TO RELEASE PARACRINE MODULATORS IN THE ENTERIC NERVOUS SYSTEM (ENS)

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Release of substance P (SP) or calcitonin gene-related peptide (CGRP) from primary afferent terminals evokes excitatory responses in ENS neurons. We tested a hypothesis that afferent release of SP and/or CGRP stimulates release of paracrine neuromodulators (e.g., histamine and proteases) from enteric mast cells in guinea pig intestine. Mast cells were identified by immunoreactivity (IR) for mast cell tryptase or chymase. A subset of tryptase-IR cells expressed IR for the neurokinin-1 (NK-1) receptor. SP- and CGRP-IR nerve fibers surrounded tryptase-IR cells. Application of SP, CGRP, capsaicin or compound 48/80 released histamine into the bathing medium as determined by ELISA. Stimulation by SP was reversed by a selective NK-1 antagonist, RP6758. The selective CGRP antagonist, $CGRP_{8-37}$ reversed the action of CGRP and the selective VR-1 receptor antagonist reversed the action of capsaicin. Electrical afferent stimulation evoked histamine and protease release. Pretreatment with an NK-1 or CGRP antagonist, but not a VR-1 antagonist, suppressed afferent-evoked release. Mast cell stabilizing drugs, cromolyn or ketotifen, reduced basal release. Either of the drugs reduced the action of SP, CGRP, capsaicin and electrical stimulation of afferents. Application of SP, CGRP or histamine evoked membrane depolarization and prolonged discharge of action potentials reminiscent of slow synaptic excitation in submucosal and myenteric neurons. Neither cromolyn nor ketotifen suppressed responses to SP, CGRP or histamine. Pretreatment with cromolyn or ketotifen suppressed slow EPSP-like responses evoked by afferent stimulation. Conclusion: firing of intramural afferents releases mast cell neuromodulators in the ENS. Supported by NIH RO1 DK37238

ORAL-01-03

EVIDENCE THAT THE GHRELIN RECEPTOR AGONIST ULIMORELIN STIMULATES SPINAL CORD DEFECATION CENTRES

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The spinal defecation centres are under-explored drug targets. Increased bowel movements were a side-effect of ulimorelin treatment of gastroparesis. Although there has been no investigation of mechanism responsible, we hypothesised a spinal cord site of action. Anaesthetised rats were used to investigate this hypothesis. Intravenous ulimorelin (1-5 mg/kg) caused a significant and prolonged (~ 1h) increase in colorectal propulsive activity and expulsion of colonic contents. This was prevented by cutting the nerves emerging from the lumbosacral cord, by the nicotinic receptor antagonist hexamethonium and by antagonists of the ghrelin receptor. The effect of intravenous ulimorelin was mimicked by direct application of ulimorelin (5 µg) to the lumbosacral spinal cord. Ulimorelin is a potent prokinetic that causes propulsive contractions of the colorectum by activating ghrelin receptors of the lumbosacral defecation centres. Its effects are long-lasting, in contrast to other colokinetics that target ghrelin receptors. (143 words)

ORAL-01-04

TREATMENT OF TNBS-COLITIS WITH BONE MARROW MESENCHYMAL STEM CELLS AND CONDITIONED MEDIUM PREVENTS ENTERIC NEUROPATHY IN GUINEA-PIGS

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Damage to the enteric nervous system (ENS) associated with Inflammatory Bowel Disease (IBD) may underlie persistent alterations in gut functions controlled by the ENS. Therefore, enteric neurons may be viable targets for novel therapies. Mesenchymal stem cells (MSCs) offer beneficial therapeutic effects for tissue repair and attenuation of neurodegenerative diseases by homing to areas of inflammation and secreting soluble bioactive factors to exert neuroprotective, anti-inflammatory, and immunomodulatory properties. MSC secretion of bioactive factors into culture medium suggest MSC-conditioned medium (CM) could provide all essential factors to repair damaged tissues. Previous studies have indicated that MSCs could be used as a therapeutic tool for the treatment of IBD, however no studies have evaluated the potential of MSCs and CM to attenuate enteric neuropathy associated with intestinal inflammation. In this investigation, we used the guinea-pig model of TNBS-induced colitis. MSC and CM were applied by enema 3 hours after TNBS. The effects of MSC and CM treatments on enteric neurons were assessed by histological and immunohistochemical analyses 6 hours, 24 hours, 3 days, and 7 days after administration. Both MSC and CM treatments prevented TNBS-associated weight loss and gross morphological damage in the colon, decreased the quantity of immune infiltrate in the colonic wall ($P<0.05$) and at the level of the myenteric ganglia ($P<0.05$), prevented loss of myenteric neurons ($P<0.05$), changes in nNOS immunoreactivity ($P<0.05$), and damage to nerve processes. These results provide strong evidence that both MSCs and CM-treatments can effectively prevent damage to the ENS caused by TNBS-induced colitis. (250 words)

ORAL-01-05

INHIBITION OF DORSOMEDIAL HYPOTHALAMUS, BUT NOT OF DORSOLATERAL PERIAQUEDUCTAL GREY, SUPPRESSES RESPIRATORY RESPONSE TO ALERTING STIMULI AND STRESS IN CONSCIOUS RATS

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Central pathways mediating cardiovascular activation in response to stress have been previously extensively investigated, yet pathways mediating respiratory activation remain unknown. We have previously demonstrated involvement of the amygdala and the prefrontal cortex in mediating respiratory activation in response to various stressors, while the current study investigates involvement of the dorsomedial hypothalamus (DMH) and the dorsolateral periaqueductal grey (PAG). 15 rats received microinjections of either saline or muscimol (200nM/200nl) into the DMH (n=8) or the PAG (n=7) via bilateral guide cannulas and then were subjected to respiratory assessment using whole-body plethysmography with presentation of 4 acoustic stimuli of varying intensity (0.5sec white noise, 60-90dB) and a 15-minute restraint stress. Acoustic stimuli evoked transient increases in respiratory rate proportional to the stimulus intensity, ranging from 152±52cpm in response to the 60dB stimulus to 329±31cpm in response to the 90dB stimulus; restraint stress elevated resting respiratory rate by 51±8.8cpm. Inhibition of the DMH abolished respiratory responses to the 60 and 70dB stimuli (p=.04 & .003) and significantly decreased responses to the 80 and 90dB stimuli by 209±81cpm (p=.018) and 164±65cpm (p=.02) respectively. Furthermore, microinjection of muscimol into the DMH abolished a respiratory rate increase evoked by restraint (p=.002). Inhibition of the PAG did not affect respiratory responses to any of the acoustic stimuli or the restraint stress (all p>.05). We conclude that integrity of the DMH is necessary for respiratory responses to both alerting and stressful stimuli, while integrity of PAG is not essential for generation of such responses in conscious rats. (250 words).

ORAL-01-06

INVESTIGATING THE ORIGINS OF CARDIAC VAGAL TONE

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Purpose: Cardiac vagal tone is a major determinant of health, but nobody knows where in the brain it originates. We are tracing its origins in the perfused rat working heart-brainstem preparation (WHBP), which shows strong cardiac vagal tone. At least two components can be identified: tonic and respiratory-phasic. The latter peaks during postinspiration. Mid-pontine transection abolishes postinspiratory drive, and has been reported also to abolish respiratory sinus arrhythmia. This suggests a supramedullary origin for respiratory-related vagal tone. The Kölliker-Fuse nucleus (KF) in the pons shows peak neuronal activity during post-inspiration and mediates the transition from inspiration to expiration. We hypothesised that this is a primary site from which respiratory-related cardiac vagal tone is produced. Methods: Juvenile rats (day 14-25) were anaesthetised with isofluorane, bisected below the diaphragm, decerebrated and perfused via the descending aorta with artificial cerebrospinal fluid. The left cardiac vagus nerve was isolated as it branches from the thoracic vagus nerve and the severed distal end recorded from. Microinjections of the $GABA_A$ receptor agonist isoguvacine were delivered to the KF bilaterally. Results: Cardiac vagal nerve activity displayed marked respiratory modulation at baseline. Bilateral injection of isoguvacine into KF transformed cardiac vagal activity into a tonic discharge, sometimes interrupted by late expiratory inhibition. Baroreceptor-evoked increases in activity were unaffected. Conclusions: The postinspiratory peak in cardiac vagal tone depends on the KF. Tonic drive and late expiratory inhibition originate elsewhere in the brainstem.

(233 words)

ORAL-01-07

INCREASED RESPIRATORY MODULATION OF SYMPATHETIC ACTIVITY VIA CATECHOLAMINERGIC C1 NEURONS DURING EARLY LIFE PARTICIPATES TO THE DEVELOPMENT OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT.

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In normotensive rats, selective inhibition or lesion of sympathoexcitatory catecholaminergic C1 neurones of the rostral ventrolateral medulla oblongata (RVLM) decrease the respiratory-related bursts of sympathetic nerve activity (SNA) and blood pressure, respectively. In the Spontaneously Hypertensive (SH) rat, increased respiratory-related bursts of SNA are proposed to contribute to the development of hypertension. We used lentiviral vectors to express the inhibitory Drosophila allatostatin receptor in C1 neurones of 21 days old, male SH and WKY rats. Ten days after injection, we used the working heart-brainstem preparation to record perfusion pressure (PP), thoracic SNA and phrenic nerve activity (PNA) during perfusion with 1µM allatostatin. Whilst PNA was similar between SH and WKY rats, SH rats exhibited significantly increased respiratory-related bursts of SNA, Traube-Hering waves and PP ($P<0.05$). Allatostatin didn't affect PNA but markedly attenuated respiratory-related bursts of SNA, decreased Traube-Hering waves and PP in both SH and WKY rats. Then, we injected the immunotoxin anti-dopamine beta-hydroxylase-saporin (anti-DBH-SAP) or its control mouse IgG-saporin into the RVLM of 21 days old male SH rats (pre-hypertensive age). 7 weeks later (hypertensive age), we implanted these rats with telemetry probes to measure blood pressure. SH rats injected with anti-DBH-SAP showed reduced blood pressure compared to control mouse IgG-saporin injected SH rats. Thus, C1 neurones are a key component of the central pathway transmitting respiratory modulation of sympathetic activity. As this modulation is increased in SH rats, targeting these cells might represent an efficient way to reduce, if not abolish, hypertension in the SH rat. (248 words).

ORAL-01-08

IDENTIFYING SITES IN THE BRAIN RESPONSIBLE FOR THE INCREASE IN MUSCLE SYMPATHETIC NERVE ACTIVITY IN OBSTRUCTIVE SLEEP APNOEA.

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Muscle sympathetic nerve activity (MSNA) is greatly elevated in patients with obstructive sleep apnoea (OSA) during normoxic daytime wakefulness. Increased MSNA is a precursor to hypertension and elevated cardiovascular morbidity and mortality. However, the mechanism underlying the high MSNA in OSA are not well understood. By recording MSNA concurrently with functional Magnetic Resonance Imaging (fMRI) we are aiming to identify the central processes responsible for the sympathoexcitation. Spontaneous fluctuations in muscle sympathetic nerve activity (MSNA) was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 18 OSA patients while lying in a 3T MRI scanner. Blood Oxygen Level Dependent (BOLD) contrast gradient echo, echo-planar images were continuously collected in a 4 s ON, 4 s OFF (200 volumes) sampling protocol. MSNA burst amplitudes were measured during the OFF periods and BOLD signal intensity was measured during the subsequent 4 s period to allow for neurovascular coupling and nerve conduction delays. Fluctuations in BOLD signal intensity covaried with the intensity of the concurrently recorded burst of MSNA. Preliminary group analysis showed positive correlations between MSNA and signal intensity in orbitofrontal and dorsomedial prefrontal cortices, and precuneus. These findings suggest that changes in the activity of suprabulbar regions may be responsible for the elevated MSNA in OSA. Ultimately, we hope to correlate the functional changes in the brain with the structural changes and thereby increase our understanding of the underlying mechanisms responsible for the sympathoexcitation associated with OSA. (238 words)

ORAL-02-01

AGE-DEPENDENT SOCIAL DEFICITS AFTER TRAUMATIC BRAIN INJURY IN YOUNG MICE

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Social dysfunction after traumatic brain injury can have significant long-term consequences on quality of life, however, our understanding of the trajectory and mechanisms underlying these deficits are limited. Based upon clinical findings, we hypothesized that a young brain may show particular vulnerability to such deficits, given the ongoing development of normal social behaviors throughout childhood and adolescence. To address this, we are investigating social outcomes after brain injury produced by a controlled cortical impactor in male mice at post-natal day (p) 21, an age approximating the toddler-aged child. We recently demonstrated that brain-injured mice show reduced social investigation, reduced social recognition and increased social dominance towards an unfamiliar male mouse, when tested at adulthood and compared to sham-operated controls (Semple et al., J Neurotrauma 2012). Extending these findings, we have recently identified impairments in sociosexual behaviors and communication. Of note, brain-injured mice show a striking reduction in scent-marking behavior towards a novel female, despite normal baseline measurements. Compared to sham controls, brain-injured mice also exhibit an abnormal profile of ultrasonic vocalizations in the presence of a female mouse or female-scented bedding. In contrast, mice which were injured at adolescence (p35) were remarkably resilient to these behavioral consequences. Together, these findings demonstrate an age-dependent vulnerability to social and communication deficits in young mice after traumatic brain injury. Ongoing and future studies will determine whether such changes are dependent upon injury severity or location. (232 words)

ORAL-02-02

IN VIVO DIFFUSION TENSOR IMAGING OF LESION SITE DEVELOPMENT AFTER SPINAL CORD INJURY.

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Development of advanced, non-invasive imaging techniques is critically important to better diagnose and study lesion site development after spinal cord injury (SCI). This study used ultra-high field diffusion tensor imaging (DTI) to quantitatively assess the spatiotemporal development of severe contusive spinal cord injury in mice (70 kdyne; $n \geq 4$). In vivo imaging was performed prior to injury, then again at 2 hours, 1 day, 3 days, 7 days, and 30 days post-SCI using a Bruker 16.4 T small animal nuclear magnetic resonance spectrometer. Cross-sectional spinal cord areas were measured in axial slices and various DTI parameters, i.e. fractional anisotropy (FA), axial diffusivity ($\lambda_{||}$) and radial diffusivity ($\lambda_{\perp}$), calculated for the dorsal column area and ventrolateral white matter. Cross-sectional area measurements revealed significant atrophy of the lesioned spinal cord in the chronic phase of SCI ($p<0.01$). Assessment of specific DTI parameters showed that the dorsal columns, i.e. the site of immediate impact, were most affected based on the rapid and persistent decrease in FA and $\lambda_{||}$. More gradual changes were observed for ventrolateral white matter, suggesting more delayed degeneration over time in a manner that may be amenable to therapeutic intervention. Post-mortem histopathological analysis revealed that $\lambda_{\perp}$ correlated closely with demyelination and astrogliosis. Changes in $\lambda_{||}$ appeared more indicative of axonal integrity, Wallerian degeneration and associated presence of macrophages. We conclude that longitudinal DTI provides an objective means to assess different aspects of pathology following contusive SCI and are currently exploring its use to detect treatment effects following therapeutic intervention. (250 words).

ORAL-02-03

SPECTRUM OF SHORT- AND LONG-TERM BRAIN PATHOLOGY AND LONG-TERM BEHAVIOURAL DEFICITS IN MALE REPEATED HYPOXIC RATS CLOSELY RESEMBLING HUMAN EXTREME PREMATURITY.

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Brain injury in the premature infant is associated with a high risk of neurodevelopmental disability. Previous small animal models of brain injury due to extreme prematurity typically fail to generate a spectrum of pathology and behaviour that closely resembles that observed in humans, even though they provide initial answers to numerous cellular, molecular and therapeutic questions. We tested the hypothesis that exposure of rats to repeated hypoxia from postnatal days (PN) 1-3 models the characteristic white matter neuropathological injury, gray matter volume loss, and memory deficits seen in children born extremely prematurely. Male Sprague-Dawley rats were exposed to repeated hypoxia or repeated normoxia from PN1-3. The absolute number of pre- and mature-oligodendrocytes, the surface area and g-ratio of myelin, the absolute volume of cerebral white and gray matter, and the absolute number of cerebral neurons was quantified stereologically. Spatial memory was investigated on a radial arm maze. Rats exposed to repeated hypoxia had a significant loss of pre-oligodendrocytes at PN4, cerebral white matter volume and myelin at PN14, cerebral cortical and striatal gray matter volume without neuronal loss at PN14, and cerebral myelin and memory deficits in adulthood. Decreased myelin was correlated with increased ADHD-like hyperactivity. This new small animal model of extreme prematurity generates a spectrum of short- and long-term pathology and behaviour that closely resembles that observed in humans (Journal of Neuroscience, 2013, 33:11863-11877). This new rat model provides a clinically relevant tool to investigate numerous cellular, molecular and therapeutic questions on brain injury due to extreme prematurity. (250 words).

ORAL-02-04

TWO DIFFERENT MODELS OF TRAUMATIC BRAIN INJURY INDUCE SIMILAR DEFICITS IN SENSORY PROCESSING IN THE IMMEDIATE POST-INJURY PERIOD.

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Traumatic brain injury (TBI) occurs when an external force damages the brain, and is often associated with complex patterns of brain abnormalities that lead to deficits in cognitive and motor function. Despite convincing human data indicating that changes in sensory processing are critical in establishing these deficits, there are few animal models in which TBI effects on sensory neuronal encoding has been studied. Here we use two different injury models to investigate TBI-induced changes in neuronal firing properties in sensory barrel cortex, using in vivo extracellular recording techniques and coordinated whisker motion to drive activity. TBI was induced using either the weight-drop impact acceleration (WDIA) method (n=8) or the fluid percussion injury (FPI) method (n=9). At 24hrs post-trauma extracellular recordings were obtained from barrel cortex in anesthetised animals, using both simple trapezoidal and complex naturalistic whisker motion to stimulate responses. Characterisation of neuronal output in both sham and injured animals revealed a suppression of excitatory responses that was distance-dependent from the site of injury. Our data highlight that post-TBI changes in sensory processing are largely similar between different types of injury, and these immediate changes in cortical responsiveness are likely precursors of TBI-induced cortical plasticity. (250 words).

ORAL-02-05

RESPONSE OF ENDOGENOUS NEURAL PROGENITOR CELLS TO TRAUMATIC SPINAL CORD INJURY.

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Endogenous neural progenitor cells (NPCs) have the potential to be manipulated in vivo as a possible strategy for repairing spinal cord injury (SCI). However, there is relatively little known about the normal response of these cells to injury. This study investigates the response of NPCs to traumatic SCI in both human and rat tissue.

Spinal cord tissue from people who died with and without a traumatic CNS injury was tested for NPC response using Nestin immunoreactivity. In rats, a mild contusion injury was used to examine the temporal and age-related responses of NPCs (anti-Nestin), neutrophils (H&E), astrocytes (anti-GFAP) and macrophages (anti-ED1).

In humans (n=41), nestin positive cells were significantly increased in response to trauma ($p<0.05$) and a positive response was seen at all levels of the spinal cord. There were no notable age related differences in this response. In rats (n=33), the response of NPCs peaked at 24 hours ($p<0.001$) gradually declining over time. Astrocytes and macrophages at the lesion edge increased over time and were highest at 6 weeks ($p<0.001$). At 2 weeks, the response of NPCs to SCI was higher in adult rat spinal cords compared to younger age groups ($p<0.01$).

The results of these studies suggest that NPCs have an increased response following traumatic SCI in both humans and rats. We have demonstrated a strong temporal pattern in adult rat SCI which was difficult to establish in the human tissue. Further studies are needed to determine age related differences at earlier time points. (246 words)

ORAL-02-06

TREATMENT WITH COMBINED EPO AND BDNF SUPPORTS HIPPOCAMPAL NEUROGENESIS AND IMPROVES FUNCTIONAL OUTCOME FOLLOWING FOCAL TBI

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Neurogenesis in the hippocampal dentate gyrus (DG) and subventricular zone (SVZ) is stimulated by traumatic brain injury (TBI), and provides a compelling target for novel therapies to improve recovery following trauma. Here, we attempted to augment specific stages of neurogenesis in a closed head injury (CHI) model of TBI, by treatment with erythropoietin (EPO), BDNF, or EPO+BDNF. Adult male C57BL/6 mice were subjected to CHI or sham-operation. All mice received BrdU (150 mg/kg x2 daily, days 1-4 post-CHI) to label proliferating cells, and were treated with EPO (days 0-11 post-CHI) and/or BDNF (days 7-11 post-CHI), or vehicle. Following twice-weekly functional assessment, brains were collected at 1,2 and 6weeks post-CHI (n=6-8). Treatment with EPO+BDNF improved behavioral ($P<0.001$) and motor ($P=0.002$) outcomes of CHI mice compared to individual factors and vehicle controls. Numbers of BrdU+ cells, DCX+ neuroblasts, and new mature BrdU/NeuN+ neurons were not affected by any treatment post-CHI in the SVZ or pericontusional cortex. However, in the DG, while no treatment affected proliferation at 1 week, EPO+BDNF increased the number of DCX+ neuroblasts at 2 weeks post-CHI by 50% ($P<0.05$ vs. vehicle), and BrdU/NeuN+ neurons at 6 weeks by 60% ($P<0.05$). These data demonstrate that a combination of EPO and BDNF, rather than either factor alone, is necessary to support post-traumatic neurogenesis. (250 words)

ORAL-02-07

PHARMACEUTICAL MODULATION OF AQUAPORINS 4 & 1 ATTENUATES CEREBRAL OEDEMA AND NEUROLOGICAL OUTCOME FOLLOWING ACUTE TRAUMATIC BRAIN INJURY

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PURPOSE: Brain swelling (oedema) after traumatic injury is life-threatening, yet current treatments remain inadequate, targeting symptoms rather than causes. Previous findings have implicated the role of aquaporin (AQP) water channels in the formation and resolution of cerebral swelling. This study used specific modulation of brain AQP channels to ascertain the effects on cerebral oedema following TBI. METHODS: Adult male Sprague-Dawley rats were injured using the impact acceleration model of TBI and administered either an AQP4 agonist or AQP4 antagonist at 30 min, 5, 12, 24 or 48 h post-injury and assessed for cerebral oedema formation via wet-weight/dry-weight methodology (n=5/group). Another subgroup of animals were administered either an AQP4 antagonist at 5 h, AQP4 agonist at 48 h or sequential treatment with both of the AQP agents before being assessed for motor functional outcome by accelerating rotarod. RESULTS: Single administration of an AQP4 antagonist at 5 h or an AQP4 agonist at 48 h significantly attenuated cerebral oedema ($p < 0.0001$) and improved motor functional outcome at 7 d ($p < 0.01$) following TBI. Sequential treatment with both of the AQP modulators however, demonstrated an even greater efficacy in ameliorating injury induced brain swelling ($p < 0.05$) and motor functional facility ($p < 0.05$). CONCLUSION: These findings suggest that an AQP4 antagonist administered early or an AQP4 agonist provided late after TBI significantly ameliorate oedema formation and improve motor functional outcome. Yet sequential administration with both of the AQP agents at optimal time points is more efficacious in attenuating secondary injuries. (250 words)

ORAL-02-08

ACTIVITY-DEPENDENT PLASTICITY INDUCES FUNCTIONAL CHANGES IN THE INJURED BRAIN

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We have previously demonstrated that TBI induces significant long-term increases in neuronal activity in supragranular layers of sensory cortex, coupled with persistent sensory deficits. Hence, we aimed to investigate whether brain plasticity induced by environmental enrichment (EE) could ameliorate abnormal neuronal and sensory function post-TBI. TBI (n=12) was induced using a standard impact/acceleration method; sham controls received surgery only (n=12). One week post-injury, TBI and Sham animals were randomly assigned housing in either isolated (housing in standard 31 x 44 x 25 cm cages) or enriched (housing in groups of 3, in 69 x 60 x 270 cm runs, filled with a variety of objects) conditions for 6-8 weeks. Then, in terminal experiments, extracellular recordings were obtained from barrel cortex neurons in response to whisker motion, including that mimicking motion in awake animals undertaking different tasks. Long-term EE exposure (6 weeks) attenuated TBI-induced hyperexcitability in layers 2/3, such that neuronal activity in TBI animals exposed to EE was restored to control levels. Little to no EE-induced changes in population neuronal responses occurred in [input] Layer 4 and [output] Layer 5. However, single cell responses demonstrated EE-induced hypoexcitation in L4 post-TBI. EE was also able to fully ameliorate sensory hypersensitivity post-TBI, although it was not found to improve motor function. Long-term enrichment post-TBI induces changes at both the population and single cell level in the sensory cortex, with EE restoring the excitation/inhibition balance in supragranular cortical layers, likely due to the role of EE in promoting cell survival. (247 words).

ORAL-03-01

ACCELERATION OF HABIT LEARNING FOLLOWNG COCAINE SENSITIZATION AND REVERSAL BY N-ACETYLCYSTEINE

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Drug addiction involves loss of control over drug-related behaviors. Psychostimulants have been show to lead to early habitual control over behaviors not restricted to drugs suggesting that the changes in decision-making capacity may be quite general. N-acetylcysteine (NAC) has been shown to restore glutamate homeostasis and reduce relapse to cocaine seeking. Here, we examined whether exposure to cocaine accelerates habit learning and whether such an effect could be prevented by co-administration of NAC. In addition, we used in-vitro electrophysiology to investigate the effects of cocaine on measures of synaptic plasticity and the ability of NAC to normalize these effects in brain regions critical for goal-directed learning. Rats received daily injections of cocaine (30 mg/kg) and/or NAC (0, 60 or 120 mg/kg) for six days and were subsequently trained to self-administer food. We then examined sensitivity to outcome devaluation in these groups. Separate groups were treated with cocaine and/or NAC prior to whole-cell patch-clamp experiments examining excitatory postsynaptic currents (EPSCs). Cocaine treatment lead to earlier control by the habit system and this effect was prevented by co-treatment with NAC. Electrophysiological experiments demonstrated increased sEPSCs and mEPSCs in the dorsomedial striatum following cocaine which were also normalized by co-treatment with NAC. These data indicate that NAC normalizes glutamatergic activity in the dorsal striatum following cocaine exposure and prevents unusually rapid habitual control of performance. Promotion of goal-directed control has potential to improve treatment outcomes in human cocaine addicts. (236 words)

ORAL-03-02

PRESENCE OF IMMATURE HIPPOCAMPAL NEURONS IN THE ADULT MOUSE BRAIN IS IMPORTANT FOR LEARNING

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A primary brain structure involved in memory processing is the hippocampus, where regulated production of new neurons (neurogenesis) underpins learning and memory. With ageing, there is a reduction in hippocampal neurogenesis, which accompanies diminished performance in behavioural tasks designed to test memory. Determining precisely how new nerve cells contribute to the maintenance of learning and memory is one of the most researched areas in neuroscience. To address the functional significance of newly born neurons, recently, we have developed a transgenic mouse model (DCX^{DTR} mice) that allowed us to specifically and reversibly delete these cells in the hippocampus (Vukovic et al., 2013, *J Neurosci*). To test the effect of depletion on learning and memory, we used the active place avoidance task, in which experimental animals must learn to avoid a defined area of a rotating test arena. Using this challenging behavioural paradigm, we found direct evidence that newly born neurons are critical for optimal acquisition of novel tasks. Specifically, DCX^{DTR} mice had greater difficulty avoiding the shock zone after diphtheria toxin treatment compared to their wild-type counterparts. As a result, they received significantly more shocks (trial day 3: 5.6±0.7 vs 11.2±1, $p<0.05$; $n=12$ per genotype). Depletion of newly born neurons also resulted in a reduced Arc expression within the dentate gyrus (16.6±1.3 vs 10.3±0.4 Arc-positive cells/mm, $n=3$ per genotype; $p<0.01$), Arc being a key regulator of synaptic plasticity. Using the DCX^{DTR} mice, we are now testing the premise whether experimentally-induced neurogenesis in the aged brain is directly associated with cognitive improvements.

ORAL-03-03

CHRONIC INTERMITTENT TOLUENE INHALATION DURING ADOLESENCE IN RATS RESULTS IN LONG-TERM GLUTAMATERGIC DYSFUNCTION AND SPECIFICALLY ALTERS REWARD BASED LEARNING

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Abuse of inhaled vapours that contain toluene is a growing problem worldwide, especially for adolescents who constitute the largest population. Consequently exposure often coincides with the maturation of crucial brain structures. The objective of this study was to employ a model of inhaled toluene exposure in adolescent rats that reflects inhalation patterns observed in humans to explore whether exposure during this period of vulnerability results in “global toxicity” or more “discrete” specific affects, and if so explore the mechanism(s) mediating these potential changes. In our paradigm, male adolescent Wistar rats (postnatal day 27) were exposed to either air (n=7-22 per cohort) or chronic intermittent toluene (CIT; n=8-23 per cohort) at 10,000ppm for 1 hour, 3 times a week for up to 4 weeks which retards weight gain ($p<0.05$). Subsequent behavioural analysis indicated that CIT during adolescence results in adaptive responses that specifically affect elements of reward based learning in an operant paradigm ($p<0.05$) while motor coordination and learning, and spatial learning both remained intact. CIT during adolescence also resulted in long-term glutamatergic dysfunction when challenged with the NMDA receptor antagonist MK801 (0.5mg/kg, ip) in adulthood ($p<0.05$). Thus, this study suggests that CIT during adolescence results in functional impairments within specific neuronal circuits, such as corticostriatal circuitry. Moreover, sustained effects on NMDA receptor signaling may contribute to toluene-induced alterations in information processing of complex behaviours. (224 words).

ORAL-03-04

'ADDICTION-LIKE' DEFICITS IN BEHAVIOR AND SYNAPTIC PLASTICITY IN OBESITY PRONE RATS

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A cardinal feature of pathological over-eating, is that although the individual can describe the negative consequences of their behaviour, they have great difficulty intervening and changing their behaviour. Indeed, many overweight individuals express a desire to limit their food consumption, yet struggle to control their intake. As such pathological overeating has the capacity to resemble an addictive disorder. Our aim was to establish whether rats prone to obesity would show addiction-like impairments in behavior and synaptic plasticity. A diet-induced obesity model was used to determine two subpopulations of rats: obesity prone and obesity resistant. These rats were then assessed for operant self-administration of high fat high sugar (HFHS) pellets on fixed and progressive ratios as well as electrophysiological measurements of long term depression (LTD) in the nucleus accumbens core. Rats prone to obesity displayed 'addiction-like' behaviour as demonstrated by higher motivation to obtain a HFHS reward as compared to obesity resistant rats ($p<0.05$) as well as increased responding during periods which signaled reward unavailability ($p<0.05$). Moreover, obesity prone rats showed deficits in the ability to induce LTD ($p<0.05$). These findings suggest that in individuals prone to obesity addiction-like impairments in the brain occur which may underlie their ability to limit intake of highly palatable food. Collectively, these data support the concept of that, akin to drugs of abuse, highly palatable food can be considered 'addictive' and provides evidence that strategies used to treat drug addiction may also have utility in treatment of the pathological overeating which often underlies obesity. (249 words)

ORAL-03-05

NEUROADAPTATIONS FOLLOWING MDMA (“ECSTASY”) SELF-ADMINISTRATION

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MDMA (“ecstasy”) is an amphetamine type stimulant that preferentially increases synaptic levels of serotonin (5-HT) via reuptake blockade and reverse transport. It is self-administered by laboratory animals but the profile of self-administration is initially quite different from self-administration of other drugs of abuse. When compared to drugs like cocaine, the latency to acquisition of MDMA self-administration is relatively long and a smaller percentage of rats meet criteria for acquisition. These differences are no longer apparent following neurotoxic 5,7 DHT lesions; all rats acquired self-administration with a short latency. These findings suggest that the large increase in synaptic 5HT initially limits MDMA self-administration. With repeated exposure to self-administered MDMA, various indices of 5HT neurotransmission are reduced and MDMA-produced increases in synaptic dopamine (DA) are increased. We have suggested that this shift underlies the development of MDMA as a reliable reinforcer. Several other neuroadaptations occur as a result of exposure to MDMA. In particular, the 5HT1b receptor became desensitized as a result of MDMA self-administration. This desensitized response might also contribute to the enhanced DAergic response to MDMA following repeated self-administration.

ORAL-03-06

EXTENDED EXPOSURE TO CAFFEINE AND SUCROSE RESULTS IN PERSISTENT CHANGES TO NEUROBIOLOGY AND AN AGE DEPENDENT RESPONSE TO ACUTE METHAMPHETAMINE CHALLENGE

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Caffeine is a psychostimulant commonly consumed with high levels of sugar. Little is known about how chronic use of these substances alters brain function or behaviour. We examined the effect of chronic exposure to caffeine, sucrose or combination on protein levels in the orbitofrontal cortex (OFC). This brain region has been implicated in neuropsychiatric conditions, including obesity and addiction behaviours. Additionally we examined whether these substances alters behavioural response to methamphetamine. Adult, and adolescent Sprague Dawley rats were treated for 26 days with water, caffeine (0.6g/L), 10% sucrose or combination. After either 1 or 6 weeks treatment free, rats were challenged with saline or methamphetamine (1mg/kg, i.p). Label free quantitative shotgun proteomic analysis of the OFC was then conducted. Treatment did not produce cross-sensitization to methamphetamine in adults at 1 week, but methamphetamine induced hyperactivity was significantly reduced in combined rats compared to those pre-treated with caffeine alone. Behavioural changes were only observed in adolescent rats following the extended drug free period. Here combined pre-treated rats showed a significantly reduced response to methamphetamine. Proteomic analyses of the OFC identified over 500 differentially expressed proteins across treatments, with many relating to CDK5, dopamine, GABA and glutamate signalling. These data provide evidence for behavioural interactions between METH-mediated locomotor activity and chronic caffeine and sucrose consumption. Long-term exposure to caffeine, sucrose or combination led to different patterns of protein expression in the OFC, suggesting that the abuse of these substances produce distinct neurobiological changes to protein systems important in behaviour and mental health. (250 Words)

ORAL-03-07

ADULT VITAMIN D DEFICIENCY AND COGNITIVE DYSFUNCTION IN A MOUSE MODEL

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Despite abundant sunlight, up to one third of adult Australians over 25 years of age are exposed to suboptimal levels of vitamin D (<50 nM). The focus of this study was on a mouse model to examine the mechanism by which Adult Vitamin D (AVD) deficiency affects cognitive function and to examine key brain regions involved with cognitive performance.

We fed adult male BALB/c mice a vitamin D-deficient diet for 10 weeks prior to testing (n=7/group). Mice were trained on the 5-choice serial reaction time task, in which they had to respond to brief pulses of light. When the light was on for a long duration (2-5 seconds) there was no association between diet and performance. However, when the stimulus was on for a brief duration (0.6-1 second), AVD-deficient mice made significantly fewer correct responses ($p<0.05$). There was no effect of AVD deficiency on the latency to collect the reward. Neurochemical analysis of the medial prefrontal cortex (mPFC) showed that AVD-deficient mice had a significant reduction in the levels of glutamine and glutamate and a significant increase in the levels of GABA and glycine.

There is epidemiological research linking low vitamin D with a range of neuropsychiatric disorders. Our research confirms that AVD deficiency is associated with sublte impairments in attentional processing and significant changes in neurochemistry within the mPFC. The model is enriching our understanding of the role of vitamin D in brain health.

ORAL-03-08

ADDITIVE EFFECTS OF PRENATAL IMMUNE ACTIVATION AND MATERNAL IRON DEFICIENCY ON NEURODEVELOPMENTAL MILESTONES AND ADULT BEHAVIOUR IN RAT OFFSPRING

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Infection and iron deficiency are common during pregnancy and studies have described altered brain development in the offspring as a result of these individual maternal exposures. Given their high global incidence, they may occur simultaneously during pregnancy. We developed the first rat model of prenatal immune activation on a background of maternal iron deficiency to determine whether these factors interact to affect neurodevelopment and adult behavior in offspring. Pregnant rats were placed on iron sufficient (IS) or iron deficient (ID) diets from E2-P7, and administered LPS or saline on E15/16. LPS administration on E15 caused greater induction of serum interleukin-6 and tumor necrosis factor-α in ID dams compared to IS dams. Offspring (P0, P7) from ID dams had reduced iron in spleen, liver and brain compared to IS, which normalized by P21. Pups from ID dams showed differences in forelimb grasp and acoustic startle, whilst pups from LPS dams displayed differences in grip ability, geotaxis reflex, cliff avoidance and acoustic startle. Adult offspring from ID dams displayed significant deficits in passive avoidance learning and pre-pulse inhibition, whilst offspring from LPS treated dams showed a significant increase in social behaviour with unfamiliar rats. Our findings show effects of prenatal LPS and maternal iron deficiency were additive, such that offspring exposed to both insults displayed more neurodevelopmental and adult behavioural abnormalities than offspring exposed to one alone. Yet surprisingly, despite the interaction during gestation on dam cytokine response, there was no interaction between factors in postnatal life, suggesting independent mechanisms of action. (250 words)

ORAL-04-01

MONITORING PD-RELEVANT PHENOTYPES IN iPSC-GENERATED NEURONS FROM A SPECTRUM OF PARKINSON'S DISEASE PATIENTS FOR DRUG SCREENING AND FOR UNDERSTANDING THE BIOLOGY OF PD

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Parkinson's Disease (PD) is a progressive neurodegenerative disorder affecting 1-2% of the population over age 65. Pathologically, PD is marked by a loss of dopaminergic neurons in the substantia nigra pars compacta region of the brain. Due to lack of access to such tissue, or availability of good animal models, iPSC-generated neurons hold promise in the development of model systems to study PD.

We have generated iPSCs from patients harboring mutations in the PARKIN and LRRK2 genes, a rare case with mutations in both the LRRK2 and GBA genes, and a patient with Multiple Systems Atrophy (MSA)--a "Parkinson's Plus Syndrome" disease with no known genetic determinants. To eliminate line-to-line variations due to genetic background, we generated a set of isogenic iPSC lines that differ at a single point in the genome using the Transcription Activator-Like (TAL) effector nuclease technology. We deleted the a-synuclein gene from the MSA line in order to understand the impact of Lewy bodies, and reverted the LRRK2 and GBA mutations back to wild type in order to better understand synergies between these mutations. These iPSC lines were differentiated to neural stem cells (NSCs), and further into dopaminergic neurons and glial cells. Using the NSCs, fluorescence-based, high-throughput compatible assays have been developed to monitor phenotypes associated with PD, including oxidative stress, metabolic activity, apoptosis, mitochondrial function, and autophagy. The long-term goal is to use these optimized assays as a platform that allows for the facile interrogation of small molecule compounds in "relieving" phenotypes associated with PD.

ORAL-04-02

TAU MEDIATES EXCITOTOXIC NEURONAL DEATH IN ALZHEIMER'S DISEASE AND STROKE

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Alzheimer's disease is characterized by deposition of the microtubule-associated protein tau in neurons, forming neurofibrillary tangle. We and others have shown that tau plays a role in neuronal dysfunction prior to its deposition. In the present study, we addressed the role of tau in acute and chronic excitotoxicity, as implicated in stroke and Alzheimer's disease at the molecular level. We used mouse models of Alzheimer's disease and stroke together with tau-deficient strains to investigate the effects of excitotoxicity on neuronal function and survival. Biochemical and histological methods were used to determine the role of individual signaling pathways in disease, driven by pathway mapping and next generation mRNA sequencing. We show that reducing tau levels prevents premature death and memory deficits in mouse models of Alzheimer's disease by interfering with NMDAR-dependent excitotoxic signaling. Furthermore, we show how the absence of tau prevents excitotoxic brain damage and immediate early gene activation in a mouse model of stroke. Taken together, we revealed that tau is critically involved in mediating excitotoxicity in chronic brain damage in Alzheimer's disease and in acute brain damage stroke, providing possibly new approaches for therapeutic intervention. (187 words).

ORAL-04-03

RESTORATION OF GASTROINTESTINAL FUNCTION IN MPTP MODEL OF PARKINSON'S DISEASE.

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In addition to motor dysfunction experienced by patients with Parkinson's Disease, non-motor symptoms including constipation are commonly experienced. These symptoms have a significant impact on quality of life and understanding their cause is important to providing effective symptom relief and developing disease modifying therapeutic strategies. Several rodent models of Parkinson's Disease have shown gastrointestinal dysfunction which has been correlated with the loss of neuronal subpopulations within the enteric nervous system. Intraperitoneal administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) caused a significant reduction in the number of dopaminergic neurons within the substantia nigra pars compacta of C57BL/6 mice. A reduction in neuronal subpopulations within the myenteric plexus of the ileum 21 days after lesioning was also detected and was concomitant with a reduction in stool frequency, indicative of digestive dysfunction. Oral administration of CuII(atsm) has been shown to be neuroprotective and restore motor performance and cognitive function to MPTP lesioned mice. In this study CuII(atsm) treatment also improved stool frequency and was correlated with restoration of neuronal subpopulations in the myenteric plexus of MPTP lesioned mice. These observations suggest that the constipation experienced by Parkinson's Disease patients may be a consequence of the loss of neuronal cell populations in conjunction with enteric glial cell reactivity within the myenteric plexus of the gastrointestinal tract and that treatments such as CuII(atsm) that are neuroprotective in the central nervous system may also provide symptom release and be disease modifying in the gastrointestinal tract. (236 words)

ORAL-04-04

THE ROLE OF ANAESTHESIA IN EXACERBATION OF AD PATHOLOGY IN A TRANSGENIC MOUSE MODEL OF AMYLOID PLAQUE DEPOSITION

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Purpose: Of substantial public health significance, surgery in older people has been linked with post-operative cognitive decline and increased risk of dementia. It is unclear if this risk relates to the use of anaesthetic agents or other factors associated with surgery. In this study we have examined the effect of repeated exposure to propofol on amyloid plaque deposition in the B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/J (APP/PS1) mouse model of Alzheimer's disease (AD). Methods: Male transgenic APP/PS1 and non-transgenic littermate mice received 200mg/Kg propofol or vehicle delivered IP at 6, 7 and 8 months of age. Depth of anesthesia was assessed using the righting reflex and vas variable between animals. Tissue was harvested at 9 months of age, stained with Thiofavine S (ThioS) to examine cortical plaque load (percentage of plaque area to total brain area), plaque number and plaque size in the cortex. Analysis was performed using student T test (N=4 animals per group). Results: ThioS labeling demonstrated a significant ($p<0.05$) increase in the plaque load of propofol treated mice (0.51 ±0.05% Mean±SEM) relative to vehicle-treated mice (0.35±0.02% Mean±SEM) with an increase also in the number of plaques but no change in average plaque size. Discussion: These data suggest that repetitive exposure to anaesthetic agents may be a risk for increased AD pathology in susceptible individuals. As the population burden of AD increases, it is important to identify factors that may affect the development of pathology in order to inform appropriate practice. (word count = 238 words).

ORAL-04-05

BACE1 REGULATES THE PROTEOLYTIC PROCESSING OF $p75^{NTR}$ VIA INTERACTING WITH ITS EXTRACELLULAR DOMAIN

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Purpose: The ectodomain fragment of $p75^{NTR}$ plays a role in Aβ aggregation and amyloid plaque formation. In this study we have investigated whether $p75^{NTR}$ shedding is regulated by BACE, the rate limiting enzyme processing APP. Method: HEK-293T cells are transfected with human BACE1 and different fragments of $p75^{NTR}$ (ICD and ECD) and then cell lysed subjected to Co-IP in order to investigate whether BACE1 interacts with $p75^{NTR}$. Results: The extracellular domain of $p75^{NTR}$ ($p75^{NTR}$ECD) interacts with BACE1. Aβ, proNGF but not NGF enhanced the interaction. $P75^{NTR}$ and BACE1 are highly colocalized in cultured cortical neurons and the colocalization is increased in response to Aβ. The level of p75ECD/FL.$P75^{NTR}$ in $BACE1^{WT}$ mice was significantly higher than $BACE1^{KO}$ while TACE enzyme is not changed, indicating BACE1 regulates $p75^{NTR}$ shedding. Transfection of BACE1 HEK-293T cells increased shedding of p75ECD in a time and dose-dependent manner. Conclusion: The BACE1 interacts with $p75^{NTR}$ in the extracellular domain and the interaction increases the shedding of the $p75^{NTR}$.Our data suggest that BACE1 may play a negative feedback role by shedding the $p75^{NTR}$ ectodomain in AD pathogenesis.

Keywords: $p75^{NTR}$, BACE1, Aβ, Alzheimer's disease

ORAL-04-06

IN VIVO ANALYSIS OF THE PRESENILINS USING ZEBRAFISH REVEALS NOVEL FUNCTIONS.

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Alzheimer's disease (AD) can be classed as familial (FAD, with early onset) or sporadic (late onset). Most FAD mutations occur in the PRESENILIN genes. These genes are required for Amyloidβ peptide (Aβ) production through their role in γ-secretase cleavage of AMYLOID PRECURSOR PROTEIN. Of the over 200 PRESENILIN mutations causing FAD only one of these, the K115Efx10 mutation of PSEN2, causes truncation of the open reading frame. If translated, the truncated protein product would resemble a naturally occurring truncated isoform of PSEN2 named PS2V that is induced by hypoxia and increased in sporadic AD brains.

A variety of evidence implicates hypoxia as a contributing factor to sporadic AD. We have shown that the mechanism regulating PS2V induction is conserved during evolution. We show that PS2V acts via full-length PRESENILIN to boost γ-secretase activity. This would increase Aβ production and Notch signalling. We showed that zebrafish possess a PS2V-like isoform, PS1IV, derived from the PSEN1 orthologous gene instead of its PSEN2 orthologue. PS2V and PS1V have diverged greatly in structure while conserving actions of stimulating γ-secretase activity and suppressing the unfolded protein response. We also show that the K115Efx10 FAD mutation is similar to PS2V in its ability to boost γ-secretase activity. Our results suggest that changes in PRESENILIN activity may underlie both FAD and sporadic AD.

We are currently using new genome editing technologies to introduce K115Efx10 and other FAD mutations into zebrafish for detailed analysis.

ORAL-04-07

VALIDATION OF P75NTR EXTRACELLULAR DOMAIN AS A THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE

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We previously found that extracellular domain of neurotrophin receptor p75 (p75NTR-ECD) is an endogenous protective factor against deposition and neurotoxicities of amyloid-beta (Abeta). In the present study, we aimed to validate p75NTR-ECD as a therapeutic target for Alzheimer's disease (AD). p75NTR-ECD levels in CSF of AD patients and healthy controls were measured. Human p75NTR-ECD gene was delivered into brain or muscle of APPswe/PS1dE9 mice via adeno-associated virus (AAV) at three months (prevention study) or nine months (treatment study) of age. The behavior and AD-type pathologies were examined at 12 months of age. We found that: (1) The levels of p75NTR-ECD were decreased in CSF of AD patients in relative to healthy controls. In comparison with wild-type littermates, p75NTR-ECD levels were decreased while expression of full p75NTR was increased in brain of APPswe/PS1dE9 mice at six, nine or twelve months of age, suggesting that p75NTR-ECD shedding is inhibited in AD brain. (2) The expression of delivered p75NTR-ECD gene was detected in neurons or muscle cells three weeks after delivery, and lasted to 12 months of age when animals were culled. Furthermore expressed human p75NTR-ECD was found to bind to Abeta plaques. (3) Both intracranial and intramuscular delivery of p75NTR-ECD gene improved behavioral performance in Morris-Water Maze and Open Field Tests, and attenuated AD-type pathologies including Abeta deposition, neurite degeneration, Tau hyperphosphorylation, microgliosis and astrocytosis in prevention and treatment studies. Our studies suggest that shedding of p75NTR-ECD is inhibited in AD brain. Restoring p75NTR-ECD level can attenuate AD-type behavior abnormality and pathologies. (250 words)

ORAL-04-08

ZINC DYSHOMEOSTASIS CAUSED BY PARKINSONISM-ASSOCIATED ATP13A2 (PARK9) DEFICIENCY UNDERLIES MITOCHONDRIAL DYSFUNCTION

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Kufor-Rakeb syndrome (KRS) is an autosomal recessive, early-onset, levodopa-responsive parkinsonism that has been associated with mutations in *ATP13A2* (*PARK9*). ATP13A2 is predicted to be a cation transporter and has been associated with several divalent metals. We previously identified mitochondrial dysfunction in KRS patient-derived fibroblasts on a background of novel pathogenic compound heterozygous mutations (c.3176T>G/c.3253delC) in *ATP13A2*.

In the current study, we examined the physiological level of intracellular free zinc ions ($[Zn^{2+}]_i$) and intracellular Zn^{2+} distribution using fluorescent Zn^{2+} dyes as well as the expression level of zinc transporters in KRS patient-derived olfactory neurosphere cultures. We also investigated the effect of elevated $[Zn^{2+}]_i$ on cell viability and mitochondrial function.

When compared to controls, patient cells displayed a low $[Zn^{2+}]_i$, altered expression of zinc transporters and impaired sequestration of Zn^{2+} into LC3-positive vesicles associated with the autophagy-lysosomal pathway, indicating zinc dyshomeostasis. Cell viability tests showed a significantly higher susceptibility to pharmacological treatments that increased $[Zn^{2+}]_i$ in patient cells compared to controls. Increased $[Zn^{2+}]_i$ was found to induce the production of reactive oxygen species and exacerbate mitochondrial dysfunction, seen as mitochondrial depolarisation, fragmentation and cell death due to ATP depletion. The toxic effect of Zn^{2+} was blocked by Zn^{2+} chelation, antioxidant treatment and promotion of mitochondrial fusion.

These findings indicate that human ATP13A2 deficiency results in zinc dyshomeostasis and mitochondrial dysfunction. Our data provides insight into the molecular mechanisms of zinc dyshomeostasis in parkinsonism and its contribution to mitochondrial dysfunction, with ATP13A2 providing a molecular link between these two distinctive aetiological factors. (249 words)

ORAL-05-01

QUANTAL CURRENT TO VOLTAGE TRANSFORMATIONS AT NEUROMUSCULAR JUNCTIONS

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Objective: At synapses, the release of neurotransmitter from individual synaptic vesicles generates small quantal currents. The currents are transformed into voltages. At neuromuscular junctions (NMJs), the transformative steps remain speculative. To gain mechanistic insights into the process, we used Drosophila to perform sophisticated genetic manipulations. We modified ultrastructure that influences key transformative steps and then assessed the physiological consequences. Methods: Nerve terminals which form the NMJs are composed of individual synaptic boutons. The boutons are enmeshed within muscle membrane specializations called the postjunctional folds that bear the postsynaptic receptors. We used NMJs with naturally occurring and genetically induced variations in synaptic vesicle size and postjunctional folds. Uniquely, we made concurrent recordings of quantal currents and voltages from individual synaptic boutons. The linear slope fitted to the points in voltage-current plots represented the resistance to current flow. We additionally employed light and electron microscopy and novel statistical methods. Results: Current, but not voltage, size directly reflected the neurotransmitter content of an individual vesicle. Voltage size instead reflected the product of current size and resistance. The size of the current was governed by vesicle size, whereas the magnitude of resistance was governed by the amount of postjunctional folds. The dlg (discs-large) gene regulated both vesicle size and the amount of postjunctional folds, whereas the lap (like AP180) gene regulated vesicle size. Conclusion: These conceptually new findings indicate that the steps underlying current to voltage transformations at neuromuscular junctions are influenced by ultrastructure. Ultrastructure is in turn under fine genetic control. (248 words)

ORAL-05-02

PERSISTENCE INCREASE IN EXCITATORY DRIVE TO OREXIN NEURONS FOLLOWING COCAINE

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Purpose

Recently we published electrophysiological and neuroanatomical evidence that contingent and non-contingent cocaine exposure induces presynaptic but not post-synaptic plasticity in LH orexin (ORX) neurons immediately following cessation of drug exposure (Yeoh et al J Physiol 2012). The present study addresses two related questions, asking: i.) do these changes persist beyond day 1 of withdrawal, and ii.) do these changes also occur in mice.

Methods

C57BL/6 (n=13) and transgenic OXGFP (n=3) mice were injected daily (7-days) with either cocaine (15mg/kg) or saline (control) and acute brain slices were prepared for miniature excitatory postsynaptic currents (mEPSCs) recordings in LH neurons after 24 hrs, 7 days or 14 days of drug abstinence.

Results

The mEPSCs frequency of LH neurons in saline (control) animals was unchanged across the time points measured post-withdrawal (16.13 ± 4.39 Hz – 24hrs, 15.05 ± 3.23 Hz - 7days, and 14.83 ± 2.96 Hz – 14 days, respectively). Importantly, cocaine treatment increased mEPSCs frequency without affecting amplitude, rise or decay time, and this enhanced excitatory drive persisted for two weeks (26.89 ± 3.30 Hz – 24hrs, 25.60 ± 7.23 Hz – 7days, and 27.63 ± 2.94 Hz – 14 days, respectively).

Conclusions

The present data confirm and extend our previous findings in rat that cocaine increases excitatory drive to LH OX neurons. Importantly, we have now shown that this drive persists for at least two weeks after cocaine withdrawal. Identifying the mechanisms responsible for this cocaine-induced plasticity onto relapse critical LH ORX neurons may be relevant to the clinical treatment of addiction.

ORAL-05-03

MODULATION OF SYNAPTIC TRANSMISSION AND NUEROEXCITABILITY BY ANGIOTENSIN II IN THE NUCLEUS OF THE SOLITARY TRACT OF MICE

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Purpose: The nucleus of the solitary tract (NTS) is a key nucleus for cardiorespiratory regulation. The NTS is the central termination site of viscero-sensory neurons. Angiotensin II type 1A receptors (AT_{1A}Rs) are located on some viscero-sensory afferent terminals as well as on a subpopulation of neurons in the NTS and play a role in blood pressure control. However, it's difficult to delineate the cellular site AT_{1A}Rs main agonist, angiotensin II (AngII) is acting. This study utilised the AT_{1A}R-green fluorescent protein (GFP) transgenic mouse to determine how NTS AT_{1A}R activity affects neuroexcitability. Method: Horizontal slices (250 µm) incorporating the NTS and solitary tract (ST) were taken from transgenic mice, where the AT_{1A}R promoter drives GFP expression. Whole-cell patch clamp recordings from GFP-expressing (AT_{1A}R+) NTS neurons were obtained during ST stimulation. Excitatory post synaptic current (EPSC) amplitude, jitter and neuron resting membrane potential (RMP) were examined before and during superfusion of AngII (2 µM) and AT_{1A}R antagonist, candesartan. Results: ST stimulation evoked predominately low jitter EPSC's (< 200 µs) (75 %, n=4). EPSC amplitude remained unchanged with application of AngII. However, RMP increased (4.5 ± 0.5 mV, n=10), and this effect were blocked by candesartan. Conclusion: The majority of AT_{1A}R expressing NTS neurons receive direct viscero-sensory afferent input and AT_{1A}R activation increases neuroexcitability of these second order neurons in the caudal region of the NTS. This indicates AngII in the NTS may modulate cardiorespiratory function at the earliest stages of autonomic reflexes. (240 words)

ORAL-05-04

DENDRITIC ACTIVITY IN CORTICAL NETWORKS

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The laminar organization of projections into the cortex results in layer 2/3 pyramidal neurons receiving feedback input onto apical and tuft dendrites and feedforward input onto basal dendrites. In vitro work has shown that these different dendritic regions can generate dendritic spikes which are mediated by either NMDA or Ca^{2+} channels. Despite being a robust mode of cellular processing in vitro, evidence for dendritic spikes and their relationship to somatic spiking has not been decisively shown in pyramidal neurons in vivo. We investigated the occurrence of dendritic spikes in L2/3 pyramidal neurons in the hindlimb somatosensory cortex of urethane anesthetized rats using single-cell Ca^{2+} imaging and patch-clamp electrophysiology in vivo. NMDA spikes occur in tuft dendrites both spontaneously and following sensory input, and have a large influence on the number of output action potentials from the neuron. Using two-photon activation of a novel intracellular caged NMDA receptor antagonist (tc-MK801), we found that NMDA spikes typically (83 ± 2 %) occur in multiple branches simultaneously. Moreover, stimulation of layer 1 fibers significantly increased the probability that sensory stimulation evoked NMDA spikes in the pyramidal cell tuft dendrites. These results demonstrate that NMDA receptors play a vital role in coupling the tuft region of the pyramidal neuron to the spike initiation zone near the cell body.

ORAL-05-05

CANNABINOIDS MODULATE QUANTAL SIZE AT THE MOUSE NEUROMUSCULAR SYNAPSE IN HEALTH AND DISEASE.

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In the CNS, endocannabinnoids negatively regulate presynaptic neurotransmitter release. Here we report a rather different effect of the cannabinoid agonist WIN-55,212 (WIN) on the mouse neuromuscular junction (NMJ). Acute application of WIN to phrenic nerve hemidiaphragm preparations resulted in a 1.6fold increase in the amplitudes of nerve-evoked endplate potentials (EPPs) and spontaneous miniature EPPs (mEPPs) with no change in quantal content. This increase in quantal amplitude was occluded by antagonists of the CB1 and CB2-receptors (AM251 and AM630). The CB2-receptor antagonist AM630 significantly increased mEPP amplitude even in the absence of WIN, suggesting a chronic endogenous cannabinoid activity at the mammalian NMJ. Quantitative confocal microscopy and Fluorescence Resonance Energy Transfer revealed no effect of WIN upon the density of acetylcholine receptors within the postsynaptic membrane. In the muscle fatigue disease Myasthenia gravis neuromuscular transmission is selectively impaired via a reduction in quantal amplitude. We therefore tested the potential of WIN to restore quantal amplitude in a mouse model of anti-Muscle Specific Kinase myasthenia gravis. A single in vivo injection of WIN acutely reversed the clinical sign of decrementing compound muscle action potentials in myasthenic mice. Furthermore, in ex-vivo diaphragm muscle preparations from myasthenic mice, WIN once more raised the amplitude of both mEPPs and EPPs. Together, this data uncovers a novel form of cannabinoid regulation of neuromuscular transmission and provides evidence of a possible feedback mechanism mediated via CB2 receptors. The results suggest that cannabinoid drugs might have potential for treating disorders such as myasthenia where neuromuscular transmission is impaired. (250 words).

ORAL-05-06

THE DYNAMIC MODULATION OF POSTSYNAPTIC GLYCINE RECEPTOR CLUSTERING

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Glycinergic synapses play an essential role in inhibitory neurotransmission in the spinal cord and the brainstem. Functional neurotransmission requires localised postsynaptic clusters of receptors. This localisation of postsynaptic glycine receptors (GlyRs) during development involves glycine-mediated depolarization. In mature neurons, however, glycine is hyperpolarizing and how functional glycine synapses are established in mature neurons is unclear. In the present study, therefore, we measured changes in alpha 1 GlyR subunit fluorescence in rodent cultured spinal cord neurons. To visualize cell-surface GlyRs, we used superecliptic pHluorin-tagged GlyRs in which the fluorescence signal is abolished at low pH. A combination of fluorescence recovery after photobleaching and fluorescence loss in photobleaching was used to quantify spatial dynamics, with GlyR diffusion further studied using quantum dot single particle tracking. The results demonstrate that GlyRs are highly mobile when they are chronically-inactivated by strychnine, a selective antagonist. In contrast, the receptor mobility decreases one hour after their activation induced by removing strychnine. Activation of GlyRs, therefore, increases the stability of GlyR fluorescence at synapses, confirmed using single particle tracking as an increased dwell time at synapses for GlyRs. The increased stability at synapses was due to decreased lateral diffusion, rather than changes in membrane insertion of GlyRs, and was associated with increases in miniature synaptic current amplitudes. Overall, our results suggest that the activation of GlyRs regulates their spatial dynamics in mature neurons, and stabilizes them at synapses to enhance functional glycinergic transmission. (236 words).

ORAL-05-07

ELECTRICAL STIMULATION ALTERS PATTERNS OF EPILEPTIFORM ACTIVITY IN THE PIRIFORM CORTEX IN VITRO

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Spontaneous epileptiform events are relatively uncommon in *in vitro* slice models of epilepsy, requiring the use of electrical stimulation to elicit hyperexcitable activity. Here we examined the effect of mild electrical stimulation on patterns of epileptiform activity measured *in vitro*, using acute slices of the primary olfactory (piriform) cortex. Two-photon functional Ca^{2+} imaging was used to monitor electrical activity in networks of neurons in 450 µm-thick parasagittal slices of the piriform cortex (PC) from 18-30 day-old C57BL6 mice. Epileptiform activity was elicited by perfusing PC slices with one of three solutions: (i) ACSF plus 4-aminopyridine (100 µM), (ii) ACSF plus picrotoxin (100 µM) or bicuculline (10 µM), and (iii) ACSF with no added Mg^{2+} and high K^{+} (0Mg/HK). Electrical stimuli were delivered via a bipolar electrode in deep layer 3. In the 0Mg/HK model, neurons in layer 2 of the PC exhibited spontaneous asynchronous electrical events (n=49 slices). After mild stimulation, synchrony increased significantly (p<0.001, n=15 slices). In contrast, in the 4-aminopyridine model, activity was significantly reduced following stimulation (n=22 slices). Finally, in the bicuculline (n=4 slices) and picrotoxin (n=4 slices) models, spontaneous activity was not observed. However, stimulation induced changes in the evoked response. In conclusion, we find that mild electrical stimulation can dramatically alter patterns of hyperexcitability in three commonly-used *in vitro* models of epilepsy. The mechanism is unknown but presumably involves some form of long-lasting synaptic plasticity. Further work on mechanisms may provide insights into why the PC is unusually epileptogenic. (244 words)

ORAL-05-08

THE ROLE OF THE NEURAL CELL ADHESION MOLECULE 2 IN REGULATION OF THE MOLECULAR COMPOSITION OF GLUTAMATERGIC SYNAPSES

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The neural cell adhesion 2 (NCAM2) belongs to the immunoglobulin superfamily of cell adhesion molecules, which plays an important role in neuronal development and formation and maintenance of synapses. NCAM2 gene is triplicated in Down syndrome, and genome wide association studies implicate NCAM2 in mental disorders. Little is known however about the functions of NCAM2 in neurons. We show that NCAM2 is a synaptic cell adhesion molecule accumulating at the excitatory glutamatergic synapses. Synaptic levels of NCAM2 increase in response to the induction of long term potentiation suggesting that it plays a role in learning and memory formation. In synapses, NCAM2 forms a complex with the glutamate receptors of AMPA and NMDA subtypes. NCAM2 also interacts with the scaffolding protein Shank and CaMKII via non-overlapping binding sites at the C-terminus of its intracellular domain. Overexpression of NCAM2 is accompanied by an increase in levels of Shank in synapses and changes in the numbers of synapses and synaptic targeting of AMPA receptors. Abnormalities in glutamatergic synapse composition may thus be a factor contributing to mental disorders associated with overexpression of NCAM2 or mutations in NCAM2 gene. (225 words).

ORAL-06-01

MOLECULAR MECHANISMS IN THE TRANSCRIPTIONAL CONTROL OF CNS MYELINATION

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Central Nervous System (CNS) function requires electrical insulation of axons by myelin, a process under tight transcriptional control. Myelin gene Regulatory Factor (MyRF) is a potential master regulator, as it is critical for the development of myelinating oligodendrocytes (Emery et al., 2009) and and myelin maintenance (Koenning et al., 2012). Whether MyRF indeed is a transcription factor is controversial, as some groups report a lack of nuclear localization of MyRF. Here, we investigate the mechanisms leading to the activation and nuclear localization of MyRF, and whether it acts as a direct transcriptional activator by binding to DNA and inducing the expression of myelin genes.

Methods

The molecular mechanisms of MyRF activation and function are investigated using in silico analysis, site-directed mutagenesis, immunohistology, luciferase transcription assays and DNA pull-down assays.

Results

MyRF is a membrane-tethered transcription factor undergoing cleavage to allow for translocation of its DNA-binding domain to the nucleus. Interestingly, this cleavage occurs via a protein domain related to the autoproteolytic intramolecular chaperone domain of viral tailspike proteins. DNA pull-down assays demonstrate that MyRF binds a DNA consensus sequence highly enriched in the vicinity of myelin genes. Using regulatory elements of myelin genes containing this consensus sequence in luciferase assays, we demonstrate that MyRF can directly promote gene expression.

Conclusion

These findings identify MyRF as a transmembrane transcription factor that undergoes autoproteolytic cleavage to directly activate myelin gene expression. Understanding the molecular mechanisms of MyRF activation may enable us to modulate its activity in the context of de- and remyelination.

(250 words).

ORAL-06-02

WIDESCALE REMODELLING OF ASTROCYTE NETWORKS IN RESPONSE TO CHRONIC STRESS INDUCED DISTURBANCE OF GLUTAMATERGIC NEUROTRANSMISSION: ADAPTIVE REORGANISATION OR EARLY PATHOLOGY?

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Exposure to chronic stress, a major risk factor in the emergence of pathological mood disturbance, significantly disrupts glutamatergic neurotransmission within key mood regulatory nuclei. While the precise mechanisms involved in governing this disturbance remain unknown, modulation of glutamatergic neurotransmission is being considered as a target for future therapeutic interventions for depression. Our research group has been interested in unravelling how astrocytes contribute to stress induced disruption of glutamatergic signalling, as astrocytes are critical to the reuptake of released glutamate and its subsequent metabolism. To examine changes in astrocytes across the forebrain we have developed a series of imaging techniques that create high resolution three-dimensional reconstructions of astrocytes and have also undertaken large-scale high throughput analyses of high resolution coronal mosaics. These techniques, utilising multiple molecular indicators (GFAP, S100b, Aldh1L1, Glutamine synthetase; Glur1; Glur2; NR1; NR2A/B and others), have yielded an exquisitely detailed picture of the significant astroglial changes induced by chronic stress. We have also now completed a time course that of these alterations. We have further determined that these alterations are not attributable to changes in inflammatory signalling as long been suspected, as we could find no significant evidence of changes in apoptosis, antigen presentation, or pro-inflammatory cytokine release. Nor is there any significant evidence of cell loss as determined by unbiased stereological assessment. The results describe how exposure to chronic stress in the rat reshapes the astroglial network, providing insight into the mechanisms that mediate the serious disturbances in glutamate signalling seen in depression.

ORAL-06-03

5-FU-INDUCED MUCOSITIS LEADS TO SPINAL GFAP EXPRESSION CHANGES IN RATS

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5-Fluorouracil (5-FU) is a widely utilised and effective chemotherapy treatment. Unfortunately 5-FU induces several dose limiting side effects, such as mucositis (gastrointestinal tract (GIT) inflammatory disorder). 5-FU also readily diffuses into the central nervous system (CNS), damaging neural progenitor cells and causing proinflammatory glial reactivity. Additional immune-to-brain paths to CNS immune engagement can also be triggered, such as systemic and central pro-inflammatory cytokine release resulting in glial activation and ultimately neuroinflammation. The predominant mechanism through which 5-FU triggers neuroinflammation has been assumed to be direct activity within the brain, with little attention paid to alternate pathways. Therefore, we investigated if 5-FU induces neuroinflammation via immune signalling pathways (neuronal VS humoral) and if glial reactivity persists longer than the mucositis-driven GIT injury. Female DA rats (*n*=8) were randomly allocated to either saline control or 5-FU (single i.p. 150mg/kg dose) groups and tissues collected at 2 time points (injury peak or recovery). CNS sections (hippocampal and T6-T9; innervated by small intestine) were isolated and astrocyte GFAP expression assessed. Acute inflammation in jejunal and ileal sections was quantified using myeloperoxidase (MPO) assay. Data expressed as mean±SEM. 5-FU reduced bodyweight (% bodyweight from baseline; $p = 2x10^{-5}$) and increased MPO activity ($p = 3x10^{-4}$) at injury peak when compared to vehicle controls. GFAP expression was reduced selectively in T6-T9 at day 8 ($p = 0.04$) compared to vehicle controls. Astrocyte GFAP expression may reflect glial dysregulation. Future studies required to clarify neuroinflammatory role in 5-FU-related cognitive impairment.

ORAL-06-04

OLIGODENDROCYTE PROGENITOR CELL PROCESS MOTILITY AND SYNAPTOGENESIS IS INFLUENCED BY LOCAL NEUROTRANSMITTER RELEASE.

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Oligodendrocyte progenitor cells (OPCs) are unique amongst glial cells. During development and adulthood they receive direct synaptic input from neurons. These axon-OPC synapses are maintained as the OPC undergoes cell division and each daughter cell “inherits” synaptic connections from the parent OPC. Also, like axo-dendritic synapses, axon-OPC synapses can be stable or transient. It is now clear that synaptic signalling can promote myelination but how axon-OPC synaptogenesis is initiated and regulated and how this process influences oligodendrocyte differentiation and myelination is unclear. Our recent time-lapse imaging experiments have demonstrated that OPC processes are highly dynamic *in vitro*, and interact transiently with axons prior to myelination. Significantly, OPC processes bear a striking resemblance to axonal growth cones, showing a complex repertoire of lamelipodial and filipodial dynamics including veiling, extension and retraction. Using an *in vitro* motility assay, we determined that OPC processes were not attracted to a gradient of brain derived neurotrophic factor. Significantly, OPC processes showed increased veiling times (27.3±0.6min) and extension (3.1±2µm/hr) in response to a glutamate (1µmol/L) gradient compared to control values (18.3±1.3min and -4.9±1.9µm/hr respectively, n=14). Our electrophysiological studies indicate that cultured OPCs express voltage-gated sodium and potassium channels, but fail to initiate true action potentials. We propose, therefore, that glutamate signalling exerts an effect on process motility via downstream calcium signalling. Understanding how neurotransmitter signalling can regulate OPC cytoskeletal reorganisation and process motility will impact on axon-OPC synaptic communication and consequently myelination. (236 words)

ORAL-06-05

THE EFFECTS OF PULSED MAGNETIC FIELDS (PMFs) ON ASTROCYTE MIGRATION AND PROLIFERATION, IN AN IN VITRO INJURY MODEL

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PMFs have been shown to safely modulate neuronal physiology. The mechanisms and the optimal conditions for PMF use are unclear and there is very little data on the effect of PMFs on astrocytes. Discovering if PMFs affect glia may help optimize conditions for PMF use in clinical settings. We examined the effects of PMFs on astrocyte migration and proliferation in an *in vitro* injury model. Primary cell cultures were established using wild-type C57B1/6J mice. A series of optimization experiments were conducted to reproducibly produce astrocyte-enriched cultures (N=3). Using this method, a scratch was made in confluent cultures and cells then received 10 minutes PMF stimulation. Custom-built coils attached to the bottom of 24-well plates were used to deliver (18mT) of 1Hz, continuous theta burst stimulation, 6-9Hz, 10 Hz or sham (no stimulation control) every 24hrs, for four days, by which time the scratch had closed (N=5). The denuded area created by the scratch was measured every 24hrs by phase-contrast imaging of live cells. The scratch area was reduced over time in all conditions ($P<0.001$). No significant difference was found between any of the PMF stimulations or control ($P=0.9238$) and there was no difference in the percentage of scratch closed between any frequency at any time point ($P=0.9724$(24hr), 0.9907(48hr), 0.548(72hr) and 0.6913(96hr)). Our data suggests that PMF stimulation has no effect on astrocyte motility in an *in vitro* scratch assay. Future studies will assess the effects on PMFs on other astrocytic functions. (241 words).

ORAL-06-06

CURCUMIN STIMULATES PROLIFERATION, MIGRATION AND PHAGOCYTIC ACTIVITY OF OLFACTORY ENSHEATHING CELLS

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One of the promising strategies for neural repair therapies is the transplantation of olfactory ensheathing cells (OECs) which are the glial cells of the olfactory system. Manipulation of the behaviour of OECs could be used to improve therapeutic outcomes and there is a need for identification of small molecules that modulate OEC biology. Curcumin is a natural polyphenol compound found in the spice turmeric, which is known for its neuro- protective properties and has been reported to promote neurogenesis and nerve regeneration, however, the effects of curcumin on glia remains largely unidentified. We evaluated the effects of curcumin on the behaviour of OECs to determine if it could be of use for enhancing the activity of glia. Curcumin at a low concentration (0.5 µM) modulated the dynamic morphology of OECs with significant increases in the length of branches and number of lamellipodia, increased the rate of migration and promoted proliferation of the cells. These changes in dynamic activity resulted in a potent 10-fold stimulation of phagocytic activity in OECs. Live cell imaging analysis showed that curcumin dramatically altered the turnover of cellular processes and lamellipodia and drove the OECs into a searching phenotype reminiscent of macrophages. These results constitute the first evidence that curcumin can modulate the behaviour of olfactory glia into a phenotype potentially more favourable for neural repair improving the therapeutic use of OECs for transplantation therapies.

ORAL-06-07

670nm LIGHT REDUCES MIGRATION, PROLIFERATION AND GLIOSIS OF HUMAN GLIA CELLS OF THE EYE IN VITRO

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The macro-glia of the eye, called Muller cells, become activated and undergo gliosis in response to retinal neuronal damage. 670nm near- infrared light has been shown to reduce retinal inflammation and photoreceptor cell death in response to retinal injury or stress. The effect of 670nm light on Muller cells, however, is not well understood. Extensive activation of Muller cells can lead to damaging scarring in the eye, therefore exploring the effect of 670nm light on Muller cells must be elucidated before considering a clinical potential. This study aimed to investigate the effects of 670nm light on Muller cell activation due to stress, by assessing migrational and proliferative activity as a result of exposure to the light An in vitro stress model was established that recapitulates the stress of retinal detachment by using scratch-wounding and serum starvation. Cell migration, proliferation and gliosis were investigated by use of cell counting, MTT assay assessment, analysis of migration rate (timed microscopy and Image J analysis) and immunocytochemistry. Scratch-wounding and growth in serum-free media activated Muller cells as indicated by up-regulation of stress responses such as cell migration, proliferation and gliosis. 670nm light treatment however, reduced cell migration, proliferation and gliosis of Muller cells under stress in vitro. These findings highlight potential therapeutic promise for 670nm light in a clinical setting. Reduced Muller cell activation may circumvent scar formation and complement the reported neuro-protective effects of 670nm light on retinal neurons. (235 words)

ORAL-06-08

THE BDNF MIMETIC TDP6 SPECIFICALLY BINDS TO TRKB AND PROMOTES OLIGODENDROCYTE MYELINATION.

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In demyelinating diseases, incomplete remyelination is a significant component of the ongoing disability. Thus, there is demonstrated need to identify strategies that directly enhance remyelination. We have previously shown that BDNF promotes CNS myelin development via activating oligodendrocyte-expressed TrkB receptors in vivo. To investigate if oligodendroglial TrkB is also required for CNS remyelination, we first examined the expression profile of oligodendroglial TrkB in mice subjected to cuprizone-induced demyelination. Immunohistochemical analysis revealed that the number of TrkB-expressing oligodendrocytes in the corpus callosum was significantly reduced in mice subjected to cuprizone (n=3). Interestingly, TrkB expression was significantly up-regulated during the post-cuprizone remyelinating period (n=3), suggesting oligodendroglial TrkB is required for remyelination. In order to specifically target TrkB activation and examine its role in remyelination, we have generated a putative TrkB agonist (TDP6) that structurally mimics the region of BDNF that binds TrkB. Our preliminary binding studies revealed that TDP6 specifically interacts with TrkB, but not with TrkA or p75NTR. Using in vitro myelination assays, we have demonstrated that TDP6 significantly enhanced myelin protein expression and the number of myelinated axonal segments in vitro (n=3). Importantly, TDP6 failed to promote myelination by TrkB-deficient OPCs, indicating that the pro-myelinating effect is dependent on oligodendroglial TrkB expression. Collectively, these results demonstrate that TDP6 is a novel BDNF mimetic that selectively targets TrkB, and promotes oligodendrocyte myelination in vitro in a TrkB dependent manner. We will extend our investigation to examine the therapeutic potential of TDP6 in promoting re-myelination in vivo. (245 words).

ORAL-07-01

SUBSTANCE P: A NOVEL TARGET IN THE TREATMENT OF CEREBRAL OEDEMA AND ELEVATED INTRACRANIAL PRESSURE FOLLOWING STROKE

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Cerebral oedema and elevated intracranial pressure (ICP) is associated with significant death and disability following stroke. Despite this, the mechanisms of swelling remain poorly understood and treatment options limited. We have previously demonstrated the involvement of substance P (SP) and neurogenic inflammation in blood-brain barrier disruption and cerebral oedema following stroke, which are ameliorated by an NK1 receptor antagonist. Furthermore, ICP is reduced following ovine traumatic brain injury with an NK1 antagonist. The aim of the present study was to assess the effect of an NK1 receptor antagonist on cerebral oedema and ICP following ovine middle cerebral artery occlusion (MCAO).

Female Merino sheep (n=30) were subject to either sham surgery or permanent MCAO under anaesthesia. ICP, blood pressure and blood gases were recorded for 24hrs after stroke induction. Animals were randomized to vehicle or NK1 antagonist treatment (1mg/kg; 1xdose at 4h; 2xdose at 4h, 9h; 3xdose at 4h, 9h, 14h). At 24hrs animals underwent magnetic resonance imaging (MRA, T1, T2, DWI).

MCA occlusion resulted in significant increases in ICP over the 24hr monitoring period. NK1 antagonist treatment reduced ICP within normal limits, with multiple doses effective in maintaining this effect. Vehicle-treated animals demonstrated marked midline shift, tonsillar herniation and cerebral oedema on MRI. NK1 antagonist-treated animals showed reduced midline shift and cerebral oedema and no evidence of tonsillar herniation.

NK1 receptor antagonists are effective in reducing ICP and abnormalities on MRI following stroke. Multiple doses are required to maintain a sustained reduction in ICP, nevertheless no rebound ICP was observed.

ORAL-07-02

CEREBRAL OEDEMA: NOT THE PRIMARY CAUSE OF ICP ELEVATION FOLLOWING ISCHAEMIC STROKE

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Elevation of intracranial pressure (ICP) resulting in further injury is a significant problem after stroke. Therapeutic hypothermia is the only effective non-reperfusion neuroprotectant in human brain ischaemia. It has been generally accepted that cerebral oedema is the primary cause of ICP elevation, despite little specific data. Our aim was to determine the importance of oedema to ICP elevation post-stroke ± hypothermia treatment. Stroke was performed on Wistar (n=12) and Sprague-Dawley rats (n=12). ICP was measured pre-stroke and 24h post-stroke. Six of each group were treated with hypothermia to 32.5°C for 2.5 hours, commencing 1 hour post-stroke. Oedema volume was assessed 24h post-stroke using the wet-dry-weight method (Wistars) or with T2 MRI scans (Sprague-Dawleys). ICP increased by 32.3 ± 6.6 mmHg 24h post-stroke in normothermic Wistars and by 50.5 ± 22 mmHg in Sprague-Dawleys (both $p<0.0001$). There was no elevation in hypothermia-treated animals (both $p<0.0001$ *vs.* normothermics). Brain-water content was non-significantly higher in the stroke hemisphere of both normothermic and hypothermic Wistars (79.5 ± 1.5% *v*. 78.4 ± 1.1%, $p=0.08$; and 78.5 ± 1.3% *v.* 78.4 ± 0.8%, $p=0.8$, respectively). Sprague-Dawleys have larger strokes, and MR measured oedema volumes were also higher, though not significantly different between normothermics and hypothermics (59 ± 44mm^3 and 38 ± 22mm^3, respectively). Hypothermia-treatment completely abolished the dramatically raised ICP seen at 24h post-stroke despite non-significant differences in oedema volumes. These data challenge the current dogma and suggest that cerebral oedema may not be the primary cause of ICP elevation and that other factors may be important. (250 words)

ORAL-07-03

NPAS4 IS UP-REGULATED IN THE CORTICOLIMBIC SYSTEM OF THE RODENT BRAIN FOLLOWING FOCAL CEREBRAL ISCHAEMIA.

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The aim of this study was to use immunohistochemistry to investigate the expression of the neural activity-dependent transcription factor Neuronal PAS domain protein 4 (Npas4) in two different rodent models of stroke; middle cerebral artery occlusion in rats and Rose Bengal-mediated photochemical ischaemia in mice. Despite the differences in the size and location of the infarcts produced by these two models, the pattern of Npas4 expression was remarkably similar across both paradigms. Npas4 was found to be rapidly, robustly and transiently up-regulated in the ipsilateral hemisphere following cerebral ischaemia. No expression was observed in the infarct core or the contralateral hemisphere. In both models the same characteristic pattern of Npas4 expression was observed in discrete corticolimbic regions which included sites that were undamaged and were some distance away from the lesion. Highest expression was seen in the frontal and piriform cortices, nucleus accumbens, ventral pallidum, amygdala, thalamus and hypothalamus. This study demonstrates that: (1) following cerebral ischaemia there is a unique induction of Npas4 expression in corticolimbic regions of the rodent brain that are linked to cognition and emotion, and (2) this distinctive pattern of expression is reproduced in two distinct models of stroke that differ vastly in the size and location of the resulting infarct. We hypothesise that Npas4 is part of a transcriptional programme that becomes activated specifically within the corticolimbic circuitry following an ischemic insult and that this aberrant expression is linked to the cognitive and emotional dysfunction associated with ischaemic insult to the brain. (248 words).

ORAL-07-04

GLYCOGEN METABOLISM IS IMPAIRED IN THE BRAIN FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS

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Glycogen is the only source of stored glucose in the brain. Its metabolism is essential when arterial glucose levels are low or when delivery is compromised such as during ischemic stroke. In the present study we explored the temporal regulation of glycogen metabolism during and after focal ischemia in rats, including its metabolic enzymes. Ischemic stroke was induced by peri-vascular application of the vasoconstrictor endothelin-1 (ET-1, 60pmol) and forebrains collected 1, 3, 6 and 24 hours after stroke induction. Equal amount of proteins from sham and stroke brain lysates were examined for glycogen levels along with the expression and activity of enzymes involved in glycogen metabolism using western blots and standard biochemical assays for enzyme activity. Following ET-1 stroke elevated glycogen levels were detected in the ipsilateral hemisphere concurrently with a concomitant reduction in the expression of glycogen debranching enzyme, and activity of glycogen phosphorylase and protein kinase A ($p<0.05$, one-way ANOVA). In contrast, expression of glycogen branching enzyme and the activity of glycogen synthase remained unaltered in either the contralateral or ipsilateral hemispheres. Altered regulation of metabolic enzymes involved in the breakdown of glycogen reduces the release of stored glucose during ischemic stroke and may contribute to increased stroke severity. Manipulation of these pathways might provide a beneficial strategy for maintaining glucose homeostasis in the stroke affected brain resulting in prolonged cellular activity and minimize post-ischemic damage. (228 words).

ORAL-07-05

EXERCISE IMPROVES LEARNING AND ELEVATES NEUROGENESIS AFTER AN ENDOTHELIN-1 HIPPOCAMPAL STROKE MODEL IN ADULT MOUSE.

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The hippocampus is vulnerable following stroke, but pools of activatable hippocampal stem/precursor cells represent novel therapeutic targets to repopulate damaged regions. We have previously shown that exercise activates latent hippocampal stem cells and therefore wanted to test if exercise would also be effective in stimulating neurogenesis and behavioural recovery following stroke. To model stroke, adult female C57Bl/6 mice received a unilateral intrahippocampal injection of vasoconstrictor Endothelin-1 or vehicle. Starting 7d post-stroke, animals were given free access to a running wheel for 21d. To assess neurogenesis, animals received a single BrdU injection 6d after running, and were sacrificed 24h later followed by immunohistochemistry and cell counts. In addition, animals were tested for ability to learn spatial cues using the active place avoidance task 6d prior to stroke and 2d and 43d post-stroke. We found that stroked animals that ran had increased BrdU-positive cell density in the stroke hemisphere and received significantly fewer shocks than stroke non-runners. Additionally, stroke runners learnt over the testing period, with shock number declining over successive trials, and most interestingly, performed at a level indistinguishable from vehicle-injected animals. This study shows that following hippocampal stroke voluntary exercise may stimulate neurogenesis and improve the ability of animals to undertake hippocampal-based learning. (203 words)

ORAL-07-06

A NEW FUNCTION FOR AN OLD ENZYME: SRC PROTEIN TYROSINE KINASE DIRECTS EXCITOTOXIC NEURONAL DEATH IN STROKE

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Excitotoxicity resulting from over-stimulation of ionotropic glutamate receptors is a major cause of neuronal death in cerebral ischemic stroke. The over-stimulated receptors exert their neurotoxic effects in part by over-activation of calpains which induce neuronal death by catalyzing limited proteolysis of specific cellular proteins. Here, we report that in cultured cortical neurons and in vivo in a rat model of focal ischemic stroke, the tyrosine kinase Src is cleaved by calpains at a site in the N-terminal unique domain. This generates a truncated Src fragment of approximately 52 kDa, which we localised predominantly to the cytosol. A synthetic cell membrane-permeable fusion peptide derived from the Src unique domain prevents calpains from cleaving Src in neurons and protects against excitotoxic neuronal death. To explore the role of the truncated Src fragment in neuronal death, we expressed a recombinant truncated Src fragment in cultured neurons and examined how it affects neuronal survival. Expression of this fragment, which lacks the unique domain, was sufficient to induce neuronal death. Furthermore, inactivation of the pro-survival kinase Akt is a key step in its neurotoxic signaling pathway. As Src maintains neuronal survival, our results implicate calpain cleavage as a molecular switch converting Src from a promoter of cell survival to a mediator of neuronal death in excitotoxicity. Additionally, our results suggest blockade of calpain cleavage of Src as a potential therapeutic strategy to minimize brain damage in ischemic stroke.

(233 words)

Ref:

Hossain et al. (2013) "A Truncated Fragment of Src Protein Kinase Generated by Calpain-Mediated Cleavage is A Mediator of Neuronal Death in Excitotoxicity" J. Biol. Chem. 288:9696

ORAL-07-07

PRE-DIFFERENTIATED STEM CELLS SHOW EVIDENCE OF SYNAPTOGENESIS IN THE STROKE AFFECTED RAT BRAIN BUT EXTENSIVE SCAR FORMATION IMPEDES FUNCTIONAL RECOVERY

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Cell based therapies to promote brain repair may offer new hope for replacing lost circuitry after stroke to improve patient recovery. We have previously reported the benefits of pre-differentiating neural progenitor cells (NPCs) into GABAergic neurons for transplant over undifferentiated NPCs. We now report the maturation of these GABAergic transplants in the severely damaged stroke brain. GABAergic neurons, undifferentiated NPCs or media alone were transplanted into the rat brain (n=10/group) at 8 predetermined stereotaxic sites 7 days after endothelin-1-induced stroke targeting both the damaged and undamaged regions. Brains were harvested 28 days post-transplant. Functional assessments revealed spontaneous recovery across all three treatment groups for all behaviour tests conducted. Immunohistochemical analysis revealed pre-differentiated cells retained their neuronal phenotype evidenced by human nuclear antigen (hNA) colabelled with beta-III-tubulin, GABA and GAD. These cells also showed further evidence of functional maturation, synaptophysin expression indicative of synaptogenesis. GABAergic cells also expressed calbindin-D28k suggesting calcium signalling events associated with neurotransmission. In contrast the majority of undifferentiated NPCs colabelled with hNA and GFAP indicating astrocyte differentiation and were localised only to the border regions. Extensive glial scarring restricts migration and neurite outgrowth of GABAergic cells within the severely damaged brain and those bordering the damage. Our results revealed pre-differentiating NPCs into neuronal cells prior to transplantation repopulates the stroke damaged brain with new neurons capable of forming synapses with calcium signalling. Strategies to reduce glial scar formation may allow communication between grafted cells and host tissue to potentially translate into significant functional recovery. (247 words)

ORAL-07-08

NEUROCHEMICAL ABNORMALITIES IN RESPIRATORY CENTRES AFTER HYPERCAPNIC HYPOXIA; IMPLICATIONS FOR OSA & SIDS.

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Introduction and Aim

Intermittent hypercapnic hypoxia (IHH) is experienced by children who have obstructive sleep apnea (OSA). It may also link the risk factors for Sudden Infant Death Syndrome (SIDS) that include prone sleep, and bedsharing. Extensive studies of neurotransmitters, receptors, and markers of apoptosis in the brainstem that have been undertaken in our laboratory after IHH exposure and in SIDS, and will be presented herein.

Method

Our two brain tissue datasets are from a piglet model of IHH and from infants who died suddenly and unexpectedly; IHH exposed piglets (n=14) were compared to controls (n=14), and SIDS cases (n=67) were compared to non-SIDS cases (n=25). Tissue was immunohistochemically stained for apoptotic markers (caspase-3 & TUNEL), NMDA receptor 1, brain derived neurotrophic factor (BDNF), its receptor TrkB, and serotonin receptor 1A (5HT1A). Neurons were quantified in eight nuclei of the caudal and rostral medulla.

Results and Conclusion

Across a number of studies, in piglets exposed to IHH & amongst SIDS infants, neurotransmitter and apoptotic markers were consistently altered in the dorsal motor nucleus of the vagus (DMNV). Markers of 5HT1A & apoptosis were altered in the nucleus of the solitary tract (NTS) & the arcuate nucleus (AN). These data will be discussed, along with the physiological implications of the abnormalities identified.

ORAL-08-01

NEURON-SPECIFIC ALTERNATIVE SPLICING OF DSCAM2 IS FUNCTIONALLY REQUIRED FOR DROSOPHILA VISUAL SYSTEM DEVELOPMENT

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Down syndrome cell adhesion molecule 2 (Dscam2) is a functionally conserved transmembrane protein expressed on the surface of neurons. Dscam2 mediates self (homophilic) binding between two opposing membranes; this binding event can induce repulsion. During *Drosophila* visual system development, two different neurons, L1 and L2, require Dscam2 homophilic repulsion for forming boundaries between repeated structures in the brain and for specifying photoreceptor synapses. However, these two neurons physically contact each other within the same nerve fibre. We demonstrate that L1 and L2 express distinct Dscam2 isoforms that cannot bind to each other, and hypothesise that each neuron requires a specific isoform for proper development. To test this, we engineered the endogenous *Dscam2* locus to express a single isoform of Dscam2 in both L1 and L2, and then assessed phenotypes at the single cell level using genetic mosaic experiments. We found that when L1 and L2 express the same Dscam2 isoform, the axon terminals of these cells become significantly constricted compared to controls, presumably due to inappropriate repulsion between these closely associated terminals. We also demonstrate that neighbouring L1 axons must express the same isoform or combination of isoforms to mediate repulsion. We conclude that L1 and L2 functionally require specific Dscam2 isoforms. This allows the same repulsive recognition molecule to function in multiple neurons within the same nerve fibre. Neuron-specific alternative splicing is therefore a mechanism for increasing the diversity of recognition molecules in the brain. (236 words)

ORAL-08-02

BACURD2 IS A NOVEL PLAYER DURING CORTICAL DEVELOPMENT, WHICH INFLUENCES THE MIGRATION AND MORPHOLOGICAL DIFFERENTIATION OF CEREBRAL CORTICAL NEURONS.

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Members of the Bacurd (BTB-domain containing adaptor for Cul3-mediated RhoA degradation) proteins are implicated in neurological disorders such as Autism Spectrum Disorders, but their functions during brain development remain poorly understood.

In this study, we describe a novel role for Bacurd2 during mouse cerebral cortical development. The forced expression and knockdown of Bacurd2 significantly disrupt the migration of cortical cells in embryonic mouse brains. Furthermore, overexpression of Bacurd2 in PC12 cells results in significant impairment of neurite formation and outgrowths. We demonstrate the protein-protein interacting terminal domains of Bacurd2 to possess complementary functions for neuritogenesis, with Rnd2 and Cul3 as interacting partners to the carboxy and amino termini of Bacurd2, respectively. Since the suppression of Rnd2 expression by RNAi impairs cortical cell migration in vivo, we were only able to rescue the migration defect of Rnd2-deficient neurons with chimeric Bacurd2 polypeptides that localise to the perinuclear region of Rnd2, but not with the wild type Bacurd2. Moreover, Rnd2—deficient neurons showed defective entry into the cortical plate when co-electroporated with a chimeric Bacurd2 construct that lacks Cul3 binding activity.

Our results highlight a novel mechanism for Cul3-Bacurd2-Rnd2 interaction that regulates morphology of neurons, as well as mediating radial migration of immature neurons during cerebral cortical development. (207 words).

ORAL-08-03

CHARACTERIZATION OF DISTINCT INTERNEURON SUBTYPES GENERATED IN HUMAN FETAL CORTICAL CULTURES

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Emerging evidence suggests that the increased number and diversity of interneurons in the human cortex is achieved from progenitors found in both the ganglionic eminence and cortical germinal zones. The contribution and subtype specification of locally generated interneurons remains unknown. Dissociated primary neurons were prepared from cortical samples ranging from 17–20 gestational weeks (GW) and cultured for 30 days in vitro (DIV). Interneurons were labelled by co-transfecting cultures with an interneuron-specific promoter expressing Cre (Dlx5/6 Cre-IRES-GFP) and reporter plasmid (pCALL-tdTomato). Cultures were analysed at 2-, 9-, 16- and 30-DIV by immunohistochemistry using interneuron subtype markers Calretinin (CR), Calbindin (CB), Parvalbumin (PV) and the proliferation marker phosphohistone H3 (pHH3). Our analysis has identified cells expressing differing combinations of CR, Dlx5/6 and CB. PV-positive cells were not observed at any stage under these culture conditions. Within the CR-positive population, no colocalizaton with pHH3 was observed at 2-DIV, which progressively increased until 16- and 30-DIV. Morphological analysis of the CR- and Dlx5/6-positive cells revealed three differing morphologies: unipolar, bipolar and multipolar. At 2-DIV, the predominant morphologies present were unipolar and bipolar whilst the majority of multipolar cells were present at 16-DIV and 30-DIV. A similar time frame of morphological maturation was observed with the Dlx5/6-positive population. This study demonstrates the utility of using Dlx5/6 as a marker for human cortical interneurons and illustrates the generation of distinct subtypes from within the cortical wall. (231 words)

ORAL-08-04

BUILDING A BRAIN: THE REGULATION OF BRAIN SIZE THROUGH THE SPATIAL CONTROL OF PTEN

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In the brain a highly regulated process governs neurogenesis, with both the timing and number of cell cycles being tightly controlled to result in the complex patterning required for normal neurological function. Pten, a major tumour suppressor protein, is a central regulator of cell growth and proliferation. Individuals bearing Pten mutations can develop a number of brain disorders including macroencephaly, seizures, mental retardation, Lhermitte–Duclos disease and autism spectrum disorders. Recent evidence has highlighted that the location of Pten is important for its function, with separate roles described for both cytoplasmic and nuclear Pten. However, little is known about the cellular transport mechanisms that control Pten location. Here we report that the E3 ligase adaptor protein Ndfip1 can function to control the ubiquitin mediated localisation of Pten. Strikingly, transgenic mice expressing Ndfip1 have a distinct brain phenotype of microencephaly. Analysis of the ventricular zone neural progenitors during development revealed a significantly increased G2/M phase of the cell cycle in Ndfip1 transgenic mice, suggesting that decreased cell proliferation was causal for the microencephaly observed. Immunohistochemical analysis showed increased nuclear Pten in the subventricular zone of Ndfip1 transgenic mice. In vitro studies determined that Ndfip1 mediates the ubiquitination of Pten, promoting its nuclear localisation that results in the regulation of cell cycle proteins cyclin D1 and Plk1. Together our findings reveal a critical pathway regulating neurogenesis that can determine brain size during development. (231 words)

ORAL-08-05

THE ROLE OF NEOGENIN IN ADULT NEUROGENESIS AND PROGENITOR CELL CYCLE

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In the adult rodent brain slowly dividing neural stem cells in the subventricular zone generate neuronal precursors, which subsequently give rise to committed neuroblasts. Newly born neuroblasts migrate along the rostral migratory stream (RMS) towards the olfactory bulb (OB) and functionally integrate into the circuitry of the OB. To ensure a continuous supply of newborn interneurons throughout life, neural precursor proliferation, differentiation and migration must be tightly coordinated. Developmentally important receptors have now emerged as key modulators of adult neurogenesis. The function of the netrin receptor, Neogenin, has not been explored in this context. Neogenin loss of function (*Neo*$^{gt/gt}$) mice exhibit reduced olfactory bulb size with a specific loss of adult-born calretinin interneurons. These observations lead to the hypothesis that Neogenin plays a role in the generation of adult born interneurons.

To determine if the loss of Neogenin affected the number of dividing neurogenic precursors, *Neo*$^{gt/gt}$ and *Neo*$^{wt/wt}$ mice were injected with a single pulse of BrdU and sacrificed 2hrs later. Tissue sections were then labeled with antibodies to Ki67 (proliferating cell marker), doublecortin (Dcx - the neuroblast marker), Pax6 (intermediate progenitor cell marker - IPCs) and BrdU. Results showed that within the *Neo*$^{gt/gt}$ RMS there was a significant increase in both BrdU+ IPCs (p = 0.0006) and BrdU+ neuroblasts (p = 0.0152). Furthermore, the cell cycle index of Dcx^{+} neuroblasts in the *Neo*$^{gt/gt}$ RMS was significantly increased (p = 0.0009). We conclude that Neogenin regulates progenitor cell cycle, thereby synchronizing progenitor proliferation within the neurogenic microenvironment of the RMS. *(250 words*

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ORAL-08-06

SINGLE CELL ANALYSIS OF THE ZEBRAFISH NERVOUS SYSTEM

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Recently, the zebrafish has emerged as a useful model organism for studying the anatomical and functional connectivity of the nervous system, owing in part to the similarities in neural circuits between zebrafish and mammals. Here, we have used genetic techniques to label and trace neurons located within the cerebellum and the thalamus individually and en masse, and have been able to identify the synaptic targets of these structures. We have found that cerebellar efferent neurons, termed eurydendroid cells, target the thalamus and the optic tectum, and that thalamic output targets the optic tectum and the habenulae. We have also confirmed the presence of presynaptic terminals from the cerebellum and the thalamus in these structures through the use of a synaptically targeted GFP. In the cerebellum, we have shown that different medial-lateral regions of the cerebellum have eurydendroid cells projecting to different targets. Of these, eurydendroid cell projections to the optic tectum were seen to be topographically organised, with more medially located eurydendroid cells projecting to the rostral tectum, and more lateral cells projecting to the caudal tectum. Similarly in the thalamus the presence of four different cell types has been observed. These cell types differ in terms of their anatomical location, as well as their synaptic targets and can be distinguished based on their morphology. These findings have likely functional implications for the cerebellum and the thalamus, which we plan to identify through the use of spatial light modulation of optogenetic proteins tied with calcium imaging of induced neural activity. (250 words).

ORAL-08-07

THE ROLE OF USP9X IN THE REGULATION OF AXON SPECIFICATION

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The establishment of axon-dendrite polarity is essential for unidirectional signal transmission and the formation of complex neural circuits. Improper axon formation and neuronal migration can lead to neurodegenerative and neurocognitive diseases. The deubiquitylating enzyme, Usp9X is highly expressed in neural progenitors in a polarised manner making it a strong candidate in the regulation of neuronal polarity. To study the role of Usp9X in early neuronal development, hippocampal neurons from both wild type and knockout mice were categorised into three stages. Stage 1 neurons extend a prominent lamella around the cell body. Stage 2 neurons extend short symmetrical neurites. During the transition from stage 2 to 3, neurite symmetry is broken and one neurite is specified to become an axon. We found that in the absence of Usp9X, neuronal progression from stage 2 to 3 was significantly delayed. Loss of Usp9X delayed both axon specification as well as axonal growth, consistent with results obtained in vivo. We are currently investigating the underlying molecular mechanisms regulated by Usp9X during the establishment of neuronal polarity. Using yeast-2-hybrid screening we identified a direct interaction between the kinesin1 motor protein, Kif5B and full length Usp9X. We observed a dramatic reduction in Kif5B protein levels in Usp9X null neurons suggesting it may play a role in Usp9X regulation of axon specification and growth. Identifying critical Usp9X substrates in neurons may have direct implications on neurodegenerative and neurocognitive diseases associated with Usp9X function, such as XLID and Parkinson's disease. (242 words).

ORAL-08-08

CALCIUM SIGNALLING MECHANISMS IN ADULT SUBVENTRICULAR ZONE-DERIVED NEURAL PRECURSOR CELLS.

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Proliferation of adult neural precursor cells (NPCs) within the subventricular zone (SVZ) is thought to be dynamically regulated through niche-derived signalling molecules, including epidermal growth factor (EGF) and fibroblast growth factors (FGF); though the precise roles of these key intracellular signalling pathways remain vaguely defined. In this study, spontaneous calcium (Ca^{2+}) oscillations were observed in 40.28 % (± 3.34) of Type B/Type C NPCs. The oscillation mechanics in these cells were investigated by the application of inositol tri-phosphate receptor (IP_3R) antagonists Xestospongin C (1µM) and 2-Aminoethoxydiphenyl borate (2-APB) (50µM) which reduced the Ca^{2+} oscillation frequency by 21.35 % (± 6.6 SE), 40.75 % (± 5.9 SE) respectively. The phospholipase C gamma (PLCγ) antagonist U-73122 (5µM) also had a significant effect on the Ca^{2+} oscillation frequency, reducing them by 49.49 % (± 5.3 SE) which suggests that PLCγ and the IP_3R are involved in the regulation of the Ca^{2+} oscillations. Further results revealed that Ca^{2+} oscillations are dependent on the presence of EGF, and that EGF stimulation increased the oscillation frequency by 43.74 % ± 13 SE and the number of oscillating cells in culture (from 26.4 % ± 3.6 SE to 44.3 % ± 4.1 SE). Furthermore, increasing the dosage of these antagonists reduced the number of 5-ethynyl-2´-deoxyuridine (EdU) labelled cells in culture, which suggests that cell proliferation may be regulated by these oscillations. The research gathered from this work will provide novel insight into a biological role for Ca^{2+} signalling in this cell type.

ORAL-09-01

OPIOID-ACETYLCHOLINE-DOPAMINE INTERPLAY MODULATES DIRECT PATHWAY NEURONS IN THE NUCLEUS ACCUMBENS SHELL TO MEDIATE CHOICE BETWEEN ACTIONS.

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A fundamental component of decision-making is the ability to extract predictive information from environmental cues to guide appropriate actions. In the laboratory, we use specific Pavlovian-instrumental transfer (PIT) to study this phenomenon, where animals, under the influence of predictive stimuli, are required to bias their choice towards a predicted outcome. In light of the recently described plastic adaptations of δ-opioid receptors (DORs) in cholinergic interneurons (CINs) of the nucleus accumbens shell (NAc-S) to promote stimulus-based choice, we sought to determine how these neuromodulatory regulations reconciled with the different populations of projection neurons in the striatum. We found that dopamine D1- (D1Rs), but not D2- (D2Rs), receptors in the NAc-S were essential for expression of choice in rats. Accordingly, drd2-eGFP mice submitted to the same procedure showed major signaling events strictly excluded from D2R-eGFP-expressing neurons, supporting a major involvement of the D1R-direct pathway in the process. In rat slice electrophysiology experiments, CINs showed sensitized firing responses to DOR agonists immediately after PIT test, and further asymmetrical pharmacological experiments revealed a cooperative involvement of both D1Rs and DORs for the expression of choice. Finally, we found that selective blockade of muscarinic M4 receptors in the NAc-S, which tightly regulate the intracellular activity of D1Rs, rescued the impairment induced by DOR manipulations, establishing a link between the opioidergic modulation in CINs and the selective recruitment of D1R-direct pathway neurons. Our results suggest a tight cooperative interplay between opioidergic, cholinergic and dopaminergic neuromodulatory systems in the NAc-S to mediate stimulus-based choice between goal-directed actions. (250 words)

ORAL-09-02

THE ROLE OF P75 NEUROTROPHIN RECEPTOR IN CHOLINERGIC BASAL FOREBRAIN STRUCTURE AND FUNCTION

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The p75 neurotrophin receptor ($p75^{NTR}$) is a transmembrane protein that controls apoptosis, cell survival and differentiation in the nervous system. $p75^{NTR}$ expression occurs widely in the developing nervous system where it mediates naturally occurring cell death. Subsequently, expression is down regulated in most brain regions except in hippocampal neural progenitors and the cholinergic basal forebrain (CBF) neurons, which continue to express $p75^{NTR}$ throughout adult life, including in their axonal terminals that innervate the hippocampus. Interestingly, loss of CBF neurons through $p75^{NTR}$ is one of the earliest pathological features of Alzheimer's disease and yet the function of $p75^{NTR}$ in this brain region in healthy adults is still unclear. In the present study we used a novel conditional $p75^{NTR}$-knockout model specific to the CBF to investigate the receptor's role in regulating CBF structure and function. We found that p75NTR – deficient mice have an increase in the size and number of cholinergic neurons without any downstream effects on hippocampal structure. Furthermore, these animals displayed an improvement in idiothetic navigation while showing no differences in allothetic spatial memory. By creating a different model in which $p75^{NTR}$ is knocked-out in nestin-expressing cells we revealed that mice lacking $p75^{NTR}$ from early stages of development show severe microcephaly, owing to a significant decrease in the number of interneurons. Our results provide a new model for a time- and cell-type specific knockout of $p75^{NTR}$ and suggest a role for the receptor in regulating CBF structure and idiothetic navigation (240 words).

ORAL-09-03

EFFECTS OF ADOLESCENT SEX HORMONE MANIPULATION ON DRUG-INDUCED HYPERACTIVITY IN THE BDNF HETEROZYGOUS MOUSE MODEL OF SCHIZOPHRENIA

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The peak onset of schizophrenia appears proximal to puberty and occurs approximately 1.4 times more frequently, as well as earlier in males than females. This suggests sex hormones play a role in mediating disease progression.

Brain-derived neurotrophic factor (BDNF) supports many neurotransmitter systems implicated in schizophrenia, and its expression is reduced in the prefrontal cortex of schizophrenic patients. We have previously shown in mice that forebrain BDNF expression correlates significantly with the pubertal rise in testosterone. Despite their confluences, interactions between BDNF and androgens are understudied in schizophrenia, particularly at the crux adolescent period. We therefore examined the effects of pre-pubescent hormone manipulation in a BDNF heterozygous (het) mouse model of psychotropic-induced hyperlocomotion (an endophenotype of schizophrenia).

Pre-pubescent male wild-type (WT) and het mice were gonadectomised and implanted with placebo, testosterone or dehydrotestosterone (DHT) pellets. Upon adulthood, amphetamine and MK801-induced hyperactivity were examined. Hormonal manipulation did not alter baseline activity in either genotype. Neither genotype nor gonadectomy affected response to MK801. However, testosterone caused a reduction in MK801-induced locomotion only in WT mice. DHT had no effect, suggesting that the testosterone effect may be mediated through local conversion to estrogen and that this mechanism is compromised in het mice. Amphetamine responses in sham controls were comparable between genotypes. Gonadectomy did not alter amphetamine-induced hyperactivity. Both testosterone and DHT reduced amphetamine responses in both genotypes.

These findings demonstrate that reduced BDNF levels exacerbate the effects of hormonal intervention on MK-801-induced hyperlocomotion, suggesting a complex interaction between BDNF, testosterone and NMDAR signalling. (250 words).

ORAL-09-04

DEVELOPMENTAL MANIPULATIONS OF THE DOPAMINE SYSTEM IN DROSOPHILA LEAD TO PERSISTENT BEHAVIOURAL ABNORMALITIES IN ADULTS

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Developmental dysfunction of the dopamine (DA) system has been implicated in the neurobiology of schizophrenia. Previously it was shown that transiently altering DA activity during *Drosophila* development changes adult behavior. This study examines whether such changes are a consequence of transiently increased synaptic release or increased cellular activity in dopaminergic neurons. The *Drosophila* DA system was manipulated using a tyrosine hydroxylase (TH) Gal4 construct. We a) manipulated vesicular release of DA using UAS-syx$^{3\text{-}69}$ b) induced depolarization of TH neurons through non-specific cation channels using UAS-TrpA1, and c) increased TH neuron excitability (UAS-TrpA1), but also blocked vesicle recycling using UAS-shibire^{ts}. These thermolabile UAS constructs allowed activation to be transient during development, depending on the housing temperature of the flies. Increasing vesicular release of DA during the late pupal stage of development led to hypolocomotion in adult flies ($P<0.0001$). Inducing depolarization of TH neurons while blocking the recycling of vesicles led to the same phenotype ($P<0.01$). Increased depolarization of TH neurons had insignificant effects on adult locomotion. HPLC analysis indicated that increased depolarization of TH neurons leads to a transient decrease in DA concentration ($P<0.01$) that is rectified by adulthood. Increasing vesicular release of DA had no significant effects on DA synthesis. These results again confirm that transient changes in TH neuron activity during development lead to behavioural changes in adults. This study suggests differential mechanisms for these adult behaviours based on the timing of TH neuron activation. This work may prove useful for the dopamine ontogeny hypothesis of schizophrenia. (248 words)

ORAL-09-05

SIGNALLING EFFECTS OF TNF-α AND ITS RECEPTORS IN MOOD-LIKE BEHAVIOUS.

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TNF-α is shown to be essential for cognitive development and plays a role in anxiety-like behaviour, partially through modulating neurotrophin expression. While we have previously shown that in young mice (3 month old) lack of TNF-α and its receptors did not affect exploratory or depression-like behaviour, it is unclear whether this role changes with ageing. This study was therefore carried out to study the effects of lack of TNF-α and its receptors on mood-like behaviours and neurotrophin expression in older mice. 6 month old TNF-KO, TNF-R1 KO and TNF-R2 KO and wild-type (WT) control mice were tested (n=14 per strain) for exploratory behaviour (Hole board exploration test, HBE) and depression-like behaviour (forced swim test, FST). Levels of NGF expression in hippocampal tissue were also measured (ELISA). TNF-KO ($P < 0.05$) and TNF-R1 KO ($P < 0.01$) mice showed impaired exploration compared to age matched WT mice. Interestingly TNF-KO and TNF-R2 KO mice had significantly lower immobility in the FST than WT mice ($P < 0.001$), indicating lower depression-like behaviour in these mice. However ELISA data showed no differences in NGF expression. It appears that signalling of TNF-α and TNF-R1 is important for maintaining normal exploratory behaviour in older mice. Furthermore lack of TNF-α and TNF-R2 in older mice appears protective against depression-like behaviour. This may be caused either by enhanced signalling of TNF-R1 in the TNF-R2 KO mice, or by chronic activation of TNF-α through TNF-R2 binding, but more work is needed to validate this. (245 words)

ORAL-09-06

DOES THE NEURONAL TRANSCRIPTION FACTOR NPAS4 PLAY A ROLE IN CLINICAL DEPRESSION?

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The neuronal and brain specific transcription factor Npas4 contains both basic-Helix-Loop-Helix (bHLH) and Per-Arnt-Sim (PAS) domains. Expression occurs in the neurogenic regions of the brain including the hippocampus. Npas4 contributes to the homeostatic balance between excitations and inhibition through the regulation of inhibitory synapses on inhibitory neurons.

Reduced Npas4 expression has significant effects on plasticity and in turn learning and memory. Stresses associated with clinical depression including social isolation, drug abuse (amphetamines, opiods), perceived stress (elevated cortisol), and reductions in serotonin, melatonin or BDNF have been demonstrated to reduced levels of Npas4 expression in the brain. Following stroke in humans depression is experience by a high proportion of patients. In rodent stroke models Npas4 expression is modulated in the brain, and we have recently reported increased expression in the corticolimbic regions of the brain.

One feature of depression is the reduced ability to experience pleasure. In an Npas4 knockout mouse model we performed a saccharin preference test on standard caged mice not subjected to stress. No statistical differences were found between knockout, wild-type or heterozygous genotypes. A cortical infarct in wild-type mice induced by the Rose-Bengal photochemical technique resulted in highly significant anhedonia ($p<0.0001$). To determine if Npas4 modulates the degree of anhedonia, the saccharin preference test will be performed on knockout animals following cortical cerebral ischaemia. Cortical ischaemic stroke by the photochemical dye Rose-Bengal in mice is a robust model for behaviour testing to determine depression-like behaviour and the pathophysiological significance of Npas4. (244 words).

ORAL-09-07

AGE-ASSOCIATED CHANGES IN THE STRIATAL CHOLINERGIC SYSTEM AND THEIR EFFECT ON MOTIVATIONAL DECLINE

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Acetylcholine (ACh) is a neurotransmitter widely distributed in the central nervous system that plays a critical role in regulating several aspects of behaviour and cognition. Considerable evidence suggests that a number of cholinergic systems undergo degenerative changes during ageing and dementia, resulting in cholinergic hypofunction at specific brain areas. These alterations in cholinergic transmission have been related to the progressive cognitive impairment observed in both Alzheimer's disease patients and healthy, elderly subjects, and can be ameliorated with anticholinesterase agents, which increase ACh in synapses. Interestingly, the brain structure with the highest content of ACh and ACh-receptors is the striatum, a subcortical nucleus that is crucial for the acquisition of goal-directed behaviours. Despite that, whether age affects cholinergic transmission in the striatum is poorly understood. Importantly, it has been proposed that dysfunction of striatal circuitry may be responsible for the diminished goal-directed behaviours of aged individuals which could be a major predisposing factor for the development of neuropsychiatric disorders, such as apathy. We therefore sought to address whether the cognitive decline leading to motivational alterations in the elderly is triggered by a progressive deterioration of the striatal cholinergic system. By using advanced fluorescence imaging analysis, we found that there is an age-associated reduction of cholinergic neuronal activity. Moreover, we assessed the behaviour of aged mice through striatal-dependent paradigms and found alterations in their performances. These results suggest that an impaired striatal cholinergic transmission may contribute to the deficits in the cognitive control of actions that are characteristic of ageing and dementia. (250 words).

ORAL-09-08

CENTRAL RELAXIN-3 RECEPTOR ACTIVATION IMPAIRS FEAR EXTINCTION IN ADULT RATS

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Central control of fear and anxiety involves limbic brain areas such as central and medial amygdala, bed nucleus of the stria terminalis, ventral hippocampus and medial prefrontal cortex. Expression of relaxin-3 and its receptor, RXFP3, in these areas suggests relaxin-3/RXFP3 signalling modulates these behaviours; and to test this possibility we analysed the effects of an RXFP3 agonist, RXFP3-A2, on conditioned fear behaviour. Adult male Sprague-Dawley rats were conditioned to tone foot-shock presentations. The next day, rats received a central acute intracerebroventricular (icv) infusion of RXFP3-A2 (5, 10 or 15 µg in 5 µl) or artificial cerebrospinal fluid (aCSF). This was followed on subsequent days by extinction training with 20 tone presentations, and extinction memory testing with 5 tone presentations. Administration of 5 and 10 µg RXFP3-A2 resulted in no significant difference in conditioned freezing behaviour compared to aCSF-treated rats. However, infusion of 15 µg RXFP3-A2 significantly increased freezing behaviour during subsequent extinction training, extinction memory testing and a long-term fear memory test 2 weeks post-infusion ($P<0.05$; two-way rmANOVA). Assessment of regional brain activation after icv infusion of 15 µg RXFP3-A2, by Fos-immunostaining, revealed significant neuronal activation in the prefrontal cortex. These data indicate that in adult rats, acute, 'global' RXFP3 activation by icv administration of RXFP3-A2, results in impairment of fear extinction, via actions in the prefrontal cortex. Together with previous findings that RXFP3-A2 infusion into central amygdala enhances fear extinction, our data suggest relaxin-3/RXFP3 systems modulate fear-related neural circuits and behaviour at multiple sites of action. (249 words).

ORAL-10-01

IMPACT OF IMMUNE GENETIC VARIABILITY, PATIENT DEMOGRAPHICS AND ENVIRONMENTAL FACTORS ON PAIN SEVERITY IN FIBROMYALGIA – A PILOT STUDY

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Chronic, neuropathic pain in fibromyalgia patients severely impacts on quality of life. Immune cells in the central nervous system play a pivotal role in the generation and maintenance of enhanced pain states via the release of inflammatory factors. Genetic variants of these factors can profoundly impact pain signalling. In addition, patient demographics and environmental triggers can also influence pain severity. This pilot study modeled the impact of genetic variability in inflammatory factors, patient demographics and environmental triggers on pain severity. One hundred and seven fibromyalgia patients were recruited. Demographics and environmental trigger information were collected, pain scores on their worst day of pain were determined on a visual analogue scale, and all patients were genotyped for immune genetic variants. Linear regression with stepwise reduction was performed to determine the impact of genetic variants, demographics and environmental triggers on pain scores. 96% of fibromyalgia patients were female, with a mean age of 57 yr. The logistic regression models revealed that 22% of pain was related to immune genetic variability (P=0.03 at *MYD88* loci), while 73% was related to demographic and environmental factors ($P=2.2x10^{-10}$, the most prominent being physical and sleep issues). As a portion of pain score was attributed to genetic variability in immune mediators, these data suggest that novel treatments that target immune pathways in pain may be beneficial in some patients. This research is ongoing. (226 words).

ORAL-10-02

THE PROTECTIVE EFFECT OF A RARE P2RX7 VARIANT WITH ABSENT FUNCTION IMPLICATES THE P2X7 RECEPTOR IN THE NEUROINFLAMMATION OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system in which the HLA DRB1*1501 allele is a major risk factor. The P2X7 receptor (OMIM 602566) is a microglial/macrophage receptor which is upregulated in MS and when activated by extracellular ATP the receptor opens a cation channel which facilitates secretion of proinflammatory cytokines. In a case-control study of two Caucasian MS cohorts from Australia and New Zealand (n = 2232) we examined the genetic associations of twelve functional polymorphisms of P2X7, including 7 common (MAF>5%) and 5 rare variants (MAF<3%). The rare variant rs28360457 coding for Arg307Gln (p.R307Q) in the P2X7 receptor was associated with MS in our combined cohort with minor allele frequency of 1.14% for MS and 2.17 % for controls (OR = 0.52, p = 0.00022). This association was still significant after correction for multiple testing (p = 0.0026). Haploview analysis showed the association of p.R307Q with MS was independent of adjoining functional SNPs. In our MS cohorts, the p.R307Q variant was associated with all clinical subtypes of MS as well as DR15 negative patients but not with DR15 positive patients. In fresh human monocytes, the P2X7-induced ethidium^{+} uptake was reduced by >95% in cells heterozygous for p.R307Q consistent with a dominant negative effect of this allele. The two-fold protection conferred by the non-functional p.R307Q implicates the fully functional P2X7 receptor in the neuroinflammation of MS.

ORAL-10-03

USP9X IS A NOVEL X-LINKED INTELLECTUAL DISABILITY GENE THAT REGULATES NEURONAL MIGRATION AND AXON GROWTH.

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USP9X encodes a substrate-specific deubiquitylating enzyme, known to regulate neural progenitor cells of the developing brain. Recently we contributed to the reporting of a large systematic re-sequencing of the X-chromosome in 209 families with non-syndromic X-Linked ID (XLID). 3 unique changes in USP9X were identified in separate families. The variants segregated with the disorder, affected highly conserved amino acids and were predicted to be deleterious in-silico. Intriguingly, these variants clustered in the C-terminal region of USP9X which binds to the XLID gene Doublecortin, a microtubule associated protein involved in neuronal migration and axon growth. To provide functional evidence that USP9X is a novel intellectual disability gene and identify potential pathogenic mechanism(s) we isolated neuronal cells from the embryonic brain of the Usp9x knock-out mouse model.

In the absence of Usp9x, neurons displayed a 43% reduction in primary axonal growth and a 42% reduction in in-vitro migration ability. Whilst over-expression of USP9X was able to partially rescue the migration and axonal defects, over-expression of the USP9X variants failed to do so. 2D-DIGE followed by mass spectrometry on cortical neurons isolated from control and knock-out embryonic brains identified 28 deregulated proteins. Identified deregulated proteins highlighted disrupted microtubule and actin dynamics in the absence of USP9X. Together these data identify USP9X as a novel XLID gene that regulates neuronal migration and axon growth. Our findings are consistent with a model wherein USP9X is required for Doublecortin function during brain development (237 words).

ORAL-10-04

GENE IDENTIFICATION IN EPILEPSY, INTELLECTUAL DISABILITY AND PSYCHIATRIC DISORDERS USING MASSIVELY PARALLEL SEQUENCING REVEALS NEW NEUROLOGICAL PATHWAYS INVOLVED IN THEIR PATHOGENESIS.

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Epilepsy is a serious neurological disorder affecting approximately 2% of the population at some stage during life. Epilepsy is heterogeneous, encompassing more than 30 different sub-syndromes, which vary in age of onset, severity and types of seizures experienced. Around 70% of epilepsy cases are thought to have a genetic basis, and more than 20 epilepsy genes have so far been identified. Through collaborations we have collected a large number of familial and sporadic cases of epilepsy in order to identify the genetic causes.

Pedigrees of familial epilepsy cases were constructed and clinical information was obtained. Genomic DNA was prepared from individuals. Where families were of sufficient size, genetic linkage analysis was carried out to identify chromosomal regions harbouring the familial mutation. Massively parallel sequencing (MPS) was employed to identify novel genetic variants towards determining the mutation of major effect. The use of MPS allowed efficient detection of all genetic variants, increasing the potential for the identification of novel genes and pathways involved in epilepsy.

Using strategies combining linkage analysis with MPS and MPS alone we have identified novel genes involved in both recessive and autosomal dominant forms of epilepsy. We have extended the gene findings in rare large families to show a broader contribution of the genes KCNT1, DEPDC5, PRRT2 and PCDH19 to epilepsy and its co-morbidities.

The application of MPS has accelerated the rate of gene discovery in epilepsy, revealing new neurological pathways involved in the pathogenesis of this disorder. These new pathways may provide novel therapeutic targets. (249 words)

ORAL-10-05

EVIDENCE OF MGLUR5 DYSREGULATION IN SCHIZOPHRENIA.

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Evidence from animal and genetic studies suggests that metabotropic glutamate receptor 5 (mGluR5) is involved in the pathophysiology of schizophrenia. However, direct evidence of altered mGluR5 in the schizophrenia brain has been lacking. The aim of this study was to determine if mGluR5, or proteins that regulate mGluR5, were altered in the schizophrenia brain, specifically in the dorsolateral prefrontal cortex (DLPFC), a brain region associated with cognitive functions. Using a large cohort of 37 schizophrenia subjects and 37 controls (obtained from the NSW Tissue Resource Centre) we examined protein levels of mGluR5 in the DLPFC (BA46). In addition we examined the protein expression of several fundamental regulators of mGluR5, including Norbin (neurochondrin), Tamalin (GRASP1), and Preso-1 (FRMPD4). These regulatory proteins ensure proper trafficking, localisation, and phosphorylation of mGluR5. mGluR5 total protein levels were significantly increased (+21%; $p<0.001$) in the DLPFC in schizophrenia subjects compared to controls. This was accompanied by large reductions in mGluR5 regulatory proteins (Norbin -37%, $p<0.001$; Tamalin -30%, $p=0.040$; Preso1 -29%, $p=0.001$). There was no association of these proteins with average lifetime antipsychotic drug exposure ($p>0.124$). For the first time, we provide robust evidence of altered regulation of mGluR5 in the schizophrenia brain and suggest there may be altered cell surface expression or disrupted mGluR5 activation in schizophrenia. These findings may have crucial implications for the use of novel mGluR5-based therapeutics. (224 words).

ORAL-10-06

DETERMINING HERITABILITY OF INFLAMMATORY BIOMARKERS IN AN AGEING POPULATION USING THE TWIN DESIGN (OLDER AUSTRALIAN TWINS STUDY)

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Elevated markers of inflammation have been associated with depression and cardiovascular disease, which presents increased rates of comorbidity in the depressed population; markers include Interleukin 6 (IL6), interleukin 8 (IL8), soluble vascular adhesion protein 1 (sVCAM-1), plasminogen activator inhibitor (PAI-1), and Serum Amyloid A (SAA))). However it is still not well-established to what extent genetic factors contribute to regulation of these inflammatory biomarkers. Our aim is to determine the heritability of these cytokines and acute phase proteins in an ageing population (Older Australian Twins Study); to establish a biomarker as an endophenotype, genetic basis must be demonstrated. Cytokines (IL6, and IL8) were measured using cytometric bead array (CBA, BD Biosciences, San Diego, USA), and acute phase proteins (sVCAM-1, PAI-1, and SAA) were measured using commercially available sandwich enzyme-linked immunosorbant assay kits (Bender Medsystems GmBH, Austria, and United States Biological, USA). The heritability of these factors was determined in a twin study of 189 monozygotic twins, and 163 dizygotic twins with a mean age of 70years (±5.12 years). Heritability estimates ranged from 0 – 35%. We also present evidence for genetic polymorphisms associated with the plasma level variations. Heritability and genetic estimates presented are modest, and indicate that genetic factors may not be of major significance in the elderly population, possibly due to naturally elevated inflammation that occurs during ageing masking genetics factors. This is of potential significance when assessing genetic risk factors in an aged population for diseases such as CVD and depression where inflammation appears to play an important role.

ORAL-10-07

THE MOLECULAR NEUROSCIENCE OF GTF2IRD1: A GENE IMPLICATED IN SOCIAL INTERACTION

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Williams-Beuren syndrome (WBS) is a genetic disease that involves a series of neurological abnormalities including excessive friendliness. Therefore, haploinsufficiency of a gene(s) located in the WBS deletion causes a lack of normal social inhibition. Duplications of the same chromosomal region have recently been implicated in social withdrawal with increased risk of autism and schizophrenia. Thus, gene copy number variations in this domain are connected to social interaction behaviour. Mapping data suggest that a transcriptional regulator discovered and characterised in our laboratory, GTF2IRD1, is a strong candidate for the causative agent. To explore the basis of GTF2IRD1's role, we generated Gtf2ird1 knockout mouse lines that show developmental defects that are similar to WBS and we are interrogating the molecular and cellular mechanisms that underpin these phenotypic abnormalities. Knockout analysis has involved a battery of behavioural testing, microarray screening and detailed expression mapping. GTF2IRD1 function has been analysed using DNA binding assays, protein- protein interaction studies and genetic manipulation of cell lines. Knockout mice showed defects in motor coordination, hyperactivity, social engagement and context-specific anxiety and we have mapped Gtf2ird1 expression to brain regions that support these phenotypes, including the cerebellum, basal ganglia and limbic system. Our microarray analysis (5KO v 5WT) has revealed a set of target genes that protein interaction studies suggest are controlled via a complex of associated chromatin-modifying factors. Our work positions GTF2IRD1 as a new epigenetic regulator of neuronal differentiation and function with important consequences for the understanding of human behaviour and psychiatric disease. (246 words)

ORAL-10-08

GAIN-OF-FUNCTION OF KCNT1 AND HUMAN EPILEPSY: GENOTYPE-PHENOTYPE CORRELATION AND PHARMACOLOGICAL REVERSAL WITH QUINIDINE

Milligan CJ[1], Li M[1], Gazina E[1], Heron SE[2,3], Nair U[1], Trager C[1], Reid CA[1], Venkat A[4,5], Younkin DP[5], Dlugos DJ[4,5], Petrovski S[6,7], Goldstein DB[6], Dibbens LM[2,3], Scheffer IE[1,8-9,], Berkovic SF[8], Petrou S[1,10-11]

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Mutations in KCNT1 have been implicated in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and epilepsy of infancy with migrating focal seizures (EIMFS). More recently, a whole exome sequencing study of epileptic encephalopathies identified an additional de novo mutation in one proband with EIMFS. A Xenopus laevis oocyte based automated two-electrode voltage-clamp assay was used to examine the impact of KCNT1 mutations. The effect of quinidine was also tested. Using quantitative RT-PCR, the relative level of mouse brain Kcnt1 mRNA expression, from postnatal day 0 through to 39, was determined. All mutations (M896I, R398Q, Y796H, R928C, R428Q, A934T and P924L) increased current amplitude and in some cases hastened (R928C; A934T) or slowed (R398Q) channel kinetics. Quinidine (300 μM) significantly blocked all mutant channels ($p<0.05$). At birth, Kcnt1 mRNA was expressed at similar levels throughout the brain. In hippocampus and thalamus this expression level remained unchanged. In contrast, cortical Kcnt1 expression increased 3-fold during the first two weeks of life, remaining constant thereafter. A similar overall increase in expression occurred in the cerebellum; however, the onset was delayed until P9. Here we demonstrate that KCNT1 mutations implicated in epilepsy cause a marked increase in function. Importantly, the magnitude of this gain-of-function correlates with the severity of the clinical presentation. Finally, exposure to quinidine significantly reduces this gain-of-function for all mutations studied. These results establish a clear direction for a targeted therapy and exemplify a translational paradigm for in vitro studies informing novel therapies in a neuropsychiatric disease. (247 words).

ORAL-11-01

PERCEIVED INDEX FINGER SPACING DECREASES OVER TIME WITH THE GRASP ILLUSION

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Voluntary movement requires use of a body representation that depends on sensory information. When the index fingers are separated vertically without vision, grasping an artificial finger with the top hand reduces perceived spacing between index fingers (Héroux et al. in press, J Physiol). We investigated the time course of this 'grasp illusion', and how vision and prior experience of grasping an artificial finger influence this illusion. Forty participants sat with their left index finger 12cm above their right. Participants reported perceived index finger spacing during a series of 10-minute trials that combined the presence or absence of vision, and the presence or absence of a passive grasp of an artificial finger. Values are mean [95% confidence interval] . For the two groups with vision (each n=10), spacing was 10.0 [8.4, 11.7] cm for the 'grasp' group and 9.7 [8.4, 11.1] cm for the 'no grasp' group; this did not change over 10min ($p>0.50$). For the two groups without vision, spacing decreased from 6.9 [3.6, 10.2] cm to 4.4 [2.5, 6.3] cm ($p=0.027$) for the 'no grasp' group and from 6.1 [3.3, 8.9] cm to 3.7 [1.0, 6.5] cm for the 'grasp' group ($p=0.004$). Spacing was greater when vision was available ($p<0.001$). In summary, the size of grasp illusion gradually increases over a period of 10 minutes. Also, the absence of vision makes the index fingers feel closer together. These data provide new information about how the proprioceptive map of the body is compiled and maintained. (244 words)

ORAL-11-02

PRINCIPAL NEURONS OF THE PIRIFORM CORTEX RESPOND DIFFERENTLY TO ODOUR PRESENTATION IN VIVO

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The piriform cortex (PC) is important for the integration of olfactory information and the formation of odour images. Two types of glutamatergic principal neurons abound in layer 2 of the PC: semilunar (SL) and superficial pyramidal (SP) cells. Previous work has shown that SL and SP cells differ significantly in their morphology, somatic laminar position, connectivity and in vitro electrophysiology. PURPOSE: In light of salient differences between the two cell types, we surmise that they will contribute differentially to the processing of olfactory information in vivo. Specifically, we hypothesise that SP cells will respond more broadly to odorants than SL cells due to their more abundant intracortical connectivity. METHODS: The anterior PC of adult C57BL/6J mice was surgically exposed and whole-cell recordings were made from layer 2 principal neurons. Up to 15 odorants were delivered at the nares of the mouse with a custom-built olfactometer. RESULTS: As expected, the amplitude of spike afterhyperpolarisation (AHP) correlated strongly with the recording depth of the cells ($r = 0.41$, $p < 0.001$, $n = 62$, Pearson's analysis); hence AHP amplitude was subsequently used as a proxy for cell identity. Consistent with our hypothesis, putative SP cells (characterised by a small AHP) were broadly excited by odorants, whereas SL cells showed more selective responses ($r = 0.31$; $p < 0.05$, $n = 46$, Pearson's analysis). CONCLUSIONS: Our findings indicate that PC principal neurons indeed respond differently to odour presentation in vivo, presumably due to a difference in intracortical connectivity. (245 words)

ORAL-11-03

EFFECTS OF PARAFLOCCULUS REMOVAL ON HYPERACTIVITY AFTER ACOUSTIC TRAUMA.

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Acoustic trauma not only causes hearing loss but also results in a variety of plastic changes in central auditory pathways, such as increased spontaneous activity. This so-called hyperactivity may be involved in the generation of tinnitus, a phantom auditory sensation. Though many animal studies have consistently shown hyperactivity in auditory centres after hearing loss, tinnitus does not always develop. It has therefore been suggested that there may be involvement of non-auditory structures performing a regulatory role bringing the increased activity in the auditory system to conscious perception. Recent evidence points to the paraflocculus of the cerebellum as having such a role. The paraflocculus receives direct input from the cochlea and is anatomically connected to central auditory structures. Therefore, we investigated the effects of paraflocculus removal on hyperactivity in guinea pig inferior colliculus 2 weeks after acoustic trauma (continuous 10 kHz tone at 124 dB SPL for 2 hours). Spontaneous activity was recorded from 477 neurons (4 animals) with paraflocculus and from 559 neurons (5 animals) with paraflocculus aspirated. Results showed a significant increase in hyperactivity ($p = 0.02$) with paraflocculus removed. These results suggest that paraflocculus exerts an inhibitory effect on hyperactivity in inferior colliculus at 2 weeks after acoustic trauma. The results are surprising in view of observations in another animal model that paraflocculus removal results in reduction of tinnitus measured behaviourally. However, these latter studies were performed at much later time-points after acoustic trauma. Studies are underway to investigate the effects of paraflocculus removal on hyperactivity at later time-points (250 words).

ORAL-11-04

SELECTIVITY OF NEURONS IN MARMOSET PRIMARY AUDITORY CORTEX FOR INTERAURAL LEVEL DIFFERENCES DEPENDS ON INTENSITY

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Interaural level differences (ILD) allow localization of high-frequency sounds along the azimuth. Binaural comparisons of ILD occur first in the brainstem, but are created de novo at many higher levels. The extent to which cells in primary auditory cortex (A1) demonstrate selectivity to ILD, particularly for complex vocalizations, remain unknown. We recorded 76 single and multi-units in A1 of 6 marmosets, anaesthetized with sufentanil and N_2O. Auditory stimuli were pure tone of characteristic frequency, and three marmoset vocalisations (Ock, Tsik and Twitter), delivered with ILDs ranging from +20dB (favouring contralateral ear) to -20dB (ipsilateral).The average binaural intensities were 30, 50 or 70dB SPL. For each stimulus, the majority of units (60-80%) were selective for ILD (ANOVA $P<0.01$), and the majority of these units (≈80%) had significant interaction effects of ILD with intensity (2-way ANOVA $P<0.01$). For each tuned unit, we identified an optimal intensity where ILD tuning was best (optimal intensity): when intensity was shifted 20dB from optimal, only 50% of cells were tuning-invariant in that: (1) they remained significantly selective to ILD, and (2) the tuning model (peak or monotonic) was consistent. This was the case for all stimulus types, regardless of vocalization or pure tone. Most non-tuning-invariant cells became non-selective to ILD at non-optimal intensities. Our results suggest a complex neuronal model where excitation along with both tuned and untuned inhibition participates in generating ILD selectivity. They also suggest intensity invariant representation of ILD is not a feature of A1, but may result from “read-outs” from V1 neurons. (250 words)

ORAL-11-05

PREDICTING INDIVIDUAL ATTENTIONAL ABILITY: IS THE SIZE OF FUNCTIONAL PRIMARY VISUAL CORTEX A POTENTIAL CORRELATE?

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Attention is required to perform a range of tasks from simple visual search to complex reading tasks. It is widely accepted that attentional selection is mediated by top-down signals from higher cortical areas to early visual areas such as the primary visual cortex (V1). It has also been reported that there is considerable variation in the surface area of V1. It is possible that this variation could impact on either the number and/or specificity of attentional feedback signals to V1 and, thereby, the efficiency of attentional mechanisms in various visual tasks. In this study, we investigated whether individual differences between humans performing attention-demanding tasks relates to the functional area of V1. We found that those with a larger representation in V1 of the central 12 degrees of the visual field, as measured using BOLD signals from fMRI, were able to perform a serial search task at a faster rate (Pearson's $R = -0.57$, $p < 0.01$). Consistent with recent suggestions of the vital role of visuo-spatial attention in reading, the speed of reading also showed a strong positive correlation with the speed of visual search. However, reading speed did not correlate with the size of V1. These results support the idea that the functional size of V1 is an important determinant of the efficiency of selective attention in simple tasks such as visual search, but its relationship with the more demanding reading task is far more complex. (237 words)

ORAL-11-06

MODULATING SELECTIVE ATTENTION IN AN INSECT NEURON

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Functional imaging, physiology and psychophysics reveal that competitive selection is an important property of visual attention. However, direct neuronal correlates of such competitive processes are scarce from all animal groups. The dragonfly is a capable hunter, rapidly selecting and capturing prey, often amidst swarms of moving distractors. We recently presented electrophysiological recordings from a dragonfly visual neuron (CSTMD1), likely to underlie this target *detection* and *selection* behavior. When this size-selective neuron is presented with a pair of moving targets, the neuron responds selectively to a single target, entirely ignoring the presence of the distractor, to within the limits of neuronal variability (r^2=0.83).

By mapping the receptive field of CSTMD1 both with, and without, a proceeding 'priming' target trajectory, we reveal regions of facilitation and inhibition that represent a locus of attention (N=7, P<0.05). Furthermore, introducing pauses between the primer and mapping target stimuli displays the evolution of this focus over time. Additionally, we varied the contrast of a priming target before the introduction of a second 'distractor' target. This unveiled the threshold at which a second target can induce a 'switch' of visual attention.

CSTMD1 is a new model system for investigating the properties of selective attention within a single neuron. By varying the parameters of multiple targets presented to the dragonfly, such as the relative salience and timing, we will further elucidate the underlying mechanisms that underlie selective attention in a neuronal network.

ORAL-11-07

INTERACTIONS BETWEEN THE ON AND OFF PATHWAYS ON THE RESPONSE OUTPUT OF GANGLION CELLS OF THE EYE

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Segregation of visual information into the ON and OFF pathways starts in the retina and is partly conserved along the visual pathways. Recent evidence (Di Marco et al., 2013), however, has demonstrated that these pathways do interact and can influence each other at the level of the ganglion cells. Extracellular recordings in several visual areas showed that the strength and time course of the spike response to stimulus of the preferred contrast is very similar to the response the offset of stimuli of the non-preferred contrast. Our measurements from retinal ganglion cells (RGCs) indicate that excitatory and inhibitory conductances show asymmetries in their behaviour during the onset of the preferred contrast stimulus and the offset of the non-preferred contrast stimulus. The aim of these experiments is to investigate which conductance properties are responsible for the generation of similar responses at stimulus onset and offset. Methods: We did dynamic clamp recordings from RGCs in whole- mount retinae of the mouse. We injected the original excitatory and inhibitory synaptic conductances recorded in response to preferred and non-preferred contrast stimuli into the cell bodies of RGCs and artificial conductances in which we shuffled their onset and offset (N = 10). Results: Injection of the original excitatory and inhibitory synaptic conductances recorded in response to preferred and non-preferred contrast produced classical spike responses. Flattening the overshoot component of the excitatory conductance recorded at the offset of the non-preferred stimulus caused a reduction in spike response. Comparisons between responses to the onset of the preferred stimulus with the offset of the non-preferred stimulus indicated that the latter produces a higher response.

ORAL-11-08

ORIENTATION ANISOTROPIES IN HUMAN EARLY VISUAL CORTEX DEPEND ON CONTRAST

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Mechanisms of orientation processing in mammalian visual cortex appear matched to the environment, such that larger populations of cells are tuned to the cardinal orientations (horizontal/vertical) than oblique orientations. Perceptually, this property appears to be manifested in poorer sensitivity to oblique compared to cardinal orientations in a variety of tasks: the so-called oblique effect. Some recent functional magnetic resonance imaging (fMRI) studies have however revealed an opposite pattern of anisotropy – namely, an increased response to the oblique orientations over the cardinals: the inverse oblique effect. This might reflect efficient coding strategies optimised to the particular diet of orientations encountered during natural viewing. Accordingly, it might be expected that the anisotropies would change as the quality/strength of the oriented stimulation changes. In two experiments, the fMRI signal at 3T was measured in retinotopically-defined regions of the human visual cortex (n=5) as a function of the orientation of a sinusoidal grating, across different stimulus contrasts (10, 30 & 100% in Experiment 1; 3 & 100% in Experiment 2). The results revealed a shift from the previously observed inverse oblique effect at high contrast to an oblique effect at low contrast. In Experiment 1, a significant orientation by contrast interaction was evident only in primary visual cortex. There was a similar pattern in Experiment 2 that extended to subsequent visual areas. The qualitative change in the orientation anisotropies as a function of contrast is consistent with the idea that early visual cortex adaptively changes its coding strategy as a function of signal-to-noise ratio (250 words).

ORAL-12-01

EPHRIN-A2 SELECTIVELY GUIDES THE MIGRATION OF INTERNEURONES INTO THE SUPRAGRANULAR LAYERS OF THE NEOCORTEX

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Neocortical interneurones are born in two regions of the ventral telencephalon; the medial ganglionic eminence (MGE) and the pre-optic area (POA). They then migrate tangentially along two separate routes to populate the neocortical layers. Interneurons from the MGE follow a deep migratory stream and enter the neocortex in layer 6 and populate infragranular layers. Interneurons from the POA travel along a superficial stream into layer 1 to populate the supragranular layers. Evidences demonstrate that the two populations express different sets of guidance molecules, including Eph/ephrin to reach the correct neocortical regions to establish appropriate connectivity and form a functional brain. Using antibody staining, we analysed the expression of the GPI-membrane bound ligand ephrin-A2 in the embryonic mouse brain at embryonic day (E) 15.5 (n=3) and E16.5 (n=2). We observed expression the ventral telencephalon, in cells emerging from the POA as well as cells located in the marginal zone and cortical plate of the developing neocortex. The expression of ephrin-A2 colocalised with the interneuronal markers Calretinin and Gad65/67. To investigate further the role of ephrin-A2, we analysed the cell composition of efnA2 KO mice neocortex (n=6) and detected a reduction of Calbindin+ interneurons in the supragranular layer. Our findings suggest that interneurons in the supragranular upper layers recruit ephrin-A2 pathway to follow the superficial migratory stream. We are now utilising in vivo RNA interference approach to further understand the role of ephrin-A2 in controlling interneuron migration in the mouse developing neocortex. (words: 240)

ORAL-12-02

RHO KINASE INHIBITION PROMOTES FUNCTIONAL IMPROVEMENT AND ALTERED NEUROGENESIS FOLLOWING TRAUMATIC BRAIN INJURY

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Following adult neurogenesis most newborn neurons fail to survive. This is likely due to a lack of maturation and integration into circuitry. Enhancing the survival of these new neurons may improve function following brain injury. Inhibition of Rho kinase is known to increase neurite outgrowth and regeneration. We have previously demonstrated that Rho kinase inhibition enhances survival of newborn neurons from the sub-ventricular zone (SVZ) and hippocampus under basal conditions. Here we examined the effect of Rho kinase inhibition on SVZ and hippocampal neurogenesis and behavioural outcome following controlled cortical impact injury. BrdU was administered to mice for 2 days prior to injury and the Rho kinase inhibitor, Y27632 (20 µM), and EdU were infused in the ipsilateral lateral ventricle for 7 days after injury, with motor and cognitive behavioural assessment at 2, 7 and 33 days post-injury. Brains were taken at 35 days for analysis. Infusion of Y27632 improved motor performance 33 days after injury with no differential effects on cognition or short term memory. While no NeuN+/EdU+ cells were found at the injury site, there was a small number of NeuN+/BrdU+ neurons, which were increased by Rho kinase inhibition. EdU+ neuron numbers were also increased in the hippocampus on the injured side. There was a decrease in EdU+ astrocytes at the injury centre but no effect on EdU+ macrophage/microglia numbers. Effects on axons are currently being examined. Therefore, Rho kinase inhibition enhances motor outcome from brain injury, possibly involving increased neuron survival and decreased reactive gliosis. (248 words)

ORAL-12-03

EARLY FATE SPECIFICATION OF PLURIPOTENT STEM CELLS IMPROVES DOPAMINERGIC DIFFERENTIATION AND ISOLATION OF PROGENITORS FOR TRANSPLANTATION INTO A RODENT MODEL OF PARKINSONISM.

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While pluripotent stem cells (PSCs) represent a potentially unlimited cell source for the purpose of transplantation, poorly specified and undifferentiated cells result in suboptimal functionally integrated grafts and risks of tumors. To overcome these limitations we have developed an improved differentiation protocol for mouse PSC and made use of a reporter line (Lmx1a-GFP), enabling isolation of high proportions of dopaminergic progenitors for the purpose of transplantation into an animal model of Parkinson's disease. By comparison to pre-existing differentiation protocols our new method, involving early fate specification without the need for stromal-derived feeder layer, results in significantly greater proportions of correctly specified DA progenitors (FoxA2+/Lmx1a+/Nurr1+) and mature DA neurons (Nurr1+/TH+/Pitx3+), as revealed by FACS analysis and immunocytochemistry. Using the LMX1a-GFP mouse reporter line and aforementioned differentiation protocol, we were able to demonstrate the benefit of transplanting FACS isolated LMX1a+ dopamine progenitors compared to Lmx1a- or unsorted cells. Lmx1a+ grafts showed enhanced numbers of DA neurons, graft innervation and significant improvements in motor function. This improved protocol enables the enrichment of dopamine progenitors for the purpose of transplantation, and additionally provides high proportions of mature dopamine neurons that may be of benefit for disease modelling and drug discovery.

ORAL-12-04

DEVELOPMENT OF MATURE BDNF-SPECIFIC SANDWICH ELISA SYSTEM

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BDNF (Brain-Derived Neurotropic Factor) plays a crucial role in the nervous system. Recent studies have shown that BDNF level in serum is related to psychiatric and other aging-related chronic diseases. The enzyme-linked immunosorbent assay (ELISA) has been widely used to measure BDNF level but this assay cannot differentiate mature BDNF from proBDNF. As the function of proBDNF is different from mature BDNF, it is necessary to establish ELISA assays specific for mature BDNF and proBDNF. The aim of this study is to establish mature BDNF-specific ELISA and measure mature BDNF level in samples. Five polyclonal or monoclonal antibodies against mature BDNF were purified and characterized by Western blot and ELISA using mature BDNF and proBDNF as antigens.B34D10 and sheep a-mature BDNF were chosen as capture and detection antibody, respectively. On the cross-reactivity test, NT-3, NT-4, NGF and proBDNF were tested at 50 ng/ml concentration and showed no cross reactivity with NT-3, NT-4 and NGF and 1.0-1.4% cross-reactivity with proBDNF. The intra- and inter-assay variability was determined at different BDNF concentrations. The result showed 1.18-7.70 % range of coefficient variations on intra assay while coefficient variation inter-assay ranged from 1.90 % to 5.64 %. Recovery test was performed using pooled human serum (n=12) and 1:80 and 1:160 dilution showed 96.68 % and 96.45 % recovery rate, respectively. The LDL (Low Detection Limit) of mature BDNF ELISA is 10-20 pg/ml. This ELISA will be a useful method to measure mature BDNF level specifically in samples with very low proBDNF cross-reactivity.

ORAL-12-05

DYNAMIC IMAGING OF NEURAL REGENERATION IN VIVO

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We aim to understand the underlying mechanisms that regulate neurogenesis and regeneration of the vertebrate brain. Zebrafish can regenerate massive damage to its brain or spinal cord. We have developed an imaging assay to dynamically study spinal cord regeneration at single cell resolution in vivo. Using this imaging assay and genetic lineage tracing after spinal cord injury we have discovered that the plasticity of the glial cells is one of the major cellular mechanisms contributing to neural recovery in zebrafish. Glial cells exhibit a dual role in neural regeneration by serving as stem cells to replenish lost cells and as scaffolds to form pioneering processes ,“glial bridges” ,which span the lesion and initiate the regeneration process. By gain and loss of function approaches we have found that Fgf signalling is a key regulator of glial plasticity after injury. The dynamic infiltration of neutrophils and macrophages is an important early step in the regeneration process. In accordance with this, we recently showed that inflammation is required and sufficient to kick start and boost the regenerative response in the zebrafish brain. We have identified an architectural transcription factor that may be an intrinsic “master” regulator in coordinating signals in injury/inflammation induced neurogenesis. The expression of this architectural transcription factor is highly upregulated shortly after injury in radial glia prior to the regenerative proliferation response and its expression is regulated by the innate immune system.

ORAL-12-06

CHARACTERISATION OF DENTAL PULP STEM CELLS IN THE AGED HUMAN TOOTH

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Stem cells isolated from the dental pulp of adult teeth (dental pulp stem cells; DPSC) have been identified as a promising candidate for a novel cellular therapy to improve disability due to stroke. The aim of this study was to investigate stem cell properties of DPSC from aged teeth. The overall purpose was to determine whether cells may be sourced for autologous transplantation to treat functional disability in human stroke patients. Putative human DPSC populations were isolated from donors aged between 13 and 90 years. DPSC from three distinct age groups (<25 years, 40-50 years, >65 years) were characterised by assessing cell proliferation, expression of surface mesenchymal and neural stem cell markers (CD73, CD90, CD105, CD146, p75) and in vitro differentiation. The neurogenic potential of DPSC was further characterised by immunofluorescence with antibodies against Nestin, Beta-III Tubulin and Glial Fibrillary Acidic Protein; and whole-cell patch clamping was performed to assess the electrophysiological properties of differentiated neurons. We demonstrated for the first time that DPSC can be isolated from human donors up to 90 years of age, with preservation of osteogenic, adipogenic and neurogenic differentiation potential. Preliminary data demonstrated that DPSC from aged teeth exhibit neural markers and suggested that these cells can form functional neurons following differentiation, similar to DPSC harvested from younger donors. These findings support the possibility that cells can be sourced from teeth of stroke patients for autologous stem cell therapy. (234 words)

ORAL-12-07

ADULT NEURAL PRECURSOR CELLS FROM THE SUBVENTRICULAR ZONE CONTRIBUTE SIGNIFICANTLY TO OLIGODENDROCYTE REGENERATION AND REMYELINATION

Authors:

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Abstract body text:

Parenchymal oligodendrocyte progenitor cells (pOPCs) are considered the principal cell type responsible for oligodendrogenesis and remyelinaton in demyelinating diseases. Recent studies have demonstrated that neural precursor cells (NPCs) from the adult subventricular zone (SVZ) can also generate new oligodendrocytes following demyelination. However, the relative contribution of NPCs versus pOPCs to remyelination is unknown. We used *in vivo* genetic fate-mapping to directly assess the behaviour of each progenitor type within the corpus callosi (CC) of mice subjected to cuprizone-induced demyelination. *Nestin-CreER^{T2}* and *Pdgfra-CreER^{T2}* transgenic mice were crossed with fluorescent Cre reporter strains to map the fate of NPCs and OPCs respectively. In cuprizone-challenged mice, substantial numbers of NPCs migrated into the demyelinated CC and contributed to oligodendrogenesis. This capacity was most prominent in rostral regions adjacent to the SVZ where NPC-derived oligodendrocytes outnumbered those generated from pOPCs by 4.6-fold, indicating that NPCs have a significant competitive advantage over pOPCs for oligodendrogenesis in this region. Sixty-two percent of all nodes of Ranvier in this region were flanked by at least one paranode generated from an NPC-derived oligodendrocyte. Moreover, myelin thickness in regions subject to significant NPC-derived oligodendrogenesis was equivalent to unchallenged controls indicating that these cells make a major contribution to restoration of normal myelin caliber. We also demonstrate that a reduced efficiency of remyelination in the caudal CC was associated with impaired maturation of oligodendrogenic NPCs. Collectively, our data define a major role for NPCs in remyelination, identifying them as a key target for enhancing myelin repair in demyelinating diseases.

(word count = 249)

ORAL-12-08

IDENTIFICATION OF NOVEL NEUROTROPHIC-LIKE FACTORS: A CANDIDATE FOR PROMOTING NERVE REGENERATION IN NEUROPATHY

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Chemotactic axon guidance has an essential role in development and for re-innervation of target tissues after neuronal injury. We aimed to determine whether the low density lipoprotein receptor-related protein 1 (LRP-1) and LRP-2 receptors mediate neurite chemoattraction, and to assess their therapeutic potential in a model of peripheral neuropathy. We found LRP-1 and LRP-2 on growth cones at the leading edge and on filopodia, suggesting that they are part of the environment-sensing machinery. E16-18 rat sensory neurons were used in a growth cone turning assay to test a range of LRP ligands for chemotactic effects *in vitro*; including metallothionein II (MTII), apolipoproteinE3, tissue plasminogen activator, alpha-2-macroglobulin (α2M), vitamin D and transthyretin. Neurites were attracted to MTII (+9.8°±1.7°, $p<0.0001$, *cf.* control -1.8°±1.1°), and repulsed from α2M (-11.9°±3.4, $P<0.01$). MTII was screened for regenerative capability in a model of small-fibre neuropathy in rats. Ten age-matched rats had topical 8% capsaicin cream and placebo cream applied to contralateral areas on the lumbar dorsum. Treated areas were given 3x weekly intradermal injections of MTII or saline. Punch and epidermal roof biopsies were harvested weekly to monitor regeneration. At 14 days, regeneration was observed in saline treated capsaicin regions, compared to contralateral control skin, however MTII treated rats had complete regeneration by 7 days. The LRP-MTII chemotactic system represents a novel, non-classical axon guidance system. MTII is a candidate disease-modifying agent for the injured nervous system.

ORAL-13-01

ILLICIT STIMULANT USE IS ASSOCIATED WITH ABNORMAL SUBSTANTIA NIGRA MORPHOLOGY IN HUMANS

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Australia and New Zealand have the highest annual prevalence of illicit stimulant use in the world, 2-5 times higher than that of Europe and North America. Use of illicit stimulant drugs such as methamphetamine, cocaine, and ecstasy can cause neurotoxicity in animals and humans but the long-term consequences are not well understood. The aim of the current study was to investigate long-lasting effects of illicit stimulant use on substantia nigra morphology in humans. We hypothesised that history of illicit stimulant use is associated with abnormal substantia nigra morphology when viewed with transcranial sonography. The area of echogenic signal at the anatomical site of the substantia nigra was measured at its greatest extent in abstinent stimulant users (n=36; 31±9 yrs) and in two groups of control subjects: non-drug users (n=29; 24±5 yrs) and cannabis users (n=12; 25±7 yrs). The area of substantia nigra echogenicity was significantly larger in the stimulant group (0.273±0.078 cm^2) than in the control (0.201±0.054 cm^2; $P<0.001$) and cannabis (0.202±0.045 cm^2; $P<0.007$) groups. 53% of stimulant users exhibited echogenicity that exceeded the 90^{th} percentile for the control group. The results of the current study suggest that individuals with a history of illicit stimulant use exhibit abnormal substantia nigra morphology. The abnormality (called 'hyperechogenicity') is a strong risk factor for developing Parkinson's disease later in life. Further research is required to determine if the observed abnormality in stimulant users is associated with a functional deficit of the nigro-striatal system

(242 words)

ORAL-13-02

HUNTING FOR THE MYSTERIOUS TETANUS TOXIN RECEPTOR

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The tetanus toxin (TeNT) is one of the three most poisonous substances on Earth. It binds to the neuromuscular junction with an extremely high affinity, but its protein receptor is still unknown.

A tripeptide was shown to bind TeNT, and we selected candidate receptors containing this motif. The peptides originating from these candidates were tested for binding in vitro and ex vivo in primary motor neuron cultures and the top candidate peptide was able to block tetanus intoxication in vivo. The peptide is masking the binding site of a potential high affinity receptor.

The protein from which the peptide originates has a neuromuscular junction specific isoform and it is taken up together with TeNT into primary motor neurons. However, it cannot mediate toxin internalisation directly as it is an extracellular matrix protein. Brain derived neurotrophic factor (BDNF) enhances both binding and internalisation of TeNT. The receptor for BDNF is TrkB, which binds to the leukocyte antigen related receptor (LAR) in a ligand dependent manner. LAR was previously shown both proteins and the dependence of this interaction on BDNF gives us a clue why TeNT binding is enhanced upon neurotrophin stimulation.

Finding the protein receptor of TeNT will enable us to find a cure for the disease and it will pave the way to the use of the toxin as a carrier for targeted delivery of therapeutics into the central nervous system. (Word count: 244)

ORAL-13-03

IN VIVO HIGH-RESOLUTION, FIBRE-OPTIC MANOMETRY CHARACTERISES MYOGENIC MOTOR PATTERNS IN THE HUMAN COLON.

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Despite its size and physiological importance, the human colon is one of the least understood organs of the body. Movements of content result from the co-ordinated contraction and relaxation of the smooth muscle cells, controlled by both myogenic and neurogenic mechanisms. In studies of the human colon the myogenic activity has not been characterised. Methods: After overnight fasting, in 12 healthy controls, a fiber-optic manometry catheter (72 sensors at 1cm intervals) was colonoscopically placed and the tip clipped at the hepatic flexure. Manometry was recorded for two hours pre and post a 700cKal meal. Previously defined neurogenic motor patterns were removed from the analysis. The remaining myogenic propagating contractions were identified by custom build software and classified as propagating in a retrograde (oral) or anterograde (anal) direction. Results: Myogenic propagating contractions were identified in all subjects; pre-meal they occurred at irregular intervals ranging between 20s – 30min. Within 5min of the start of the meal a rhythmic pattern of propagating myogenic contractions (2.6 ± 0.4/min) commenced and this lasted ~30 min. During this period myogenic activity propagated in a pre-dominantly retrograde direction (retrograde (mean ± SEM), 180 ± 19 vs anterograde 110 ± 13 per/30 min; P = 0.002), with the majority of the activity occurring in the descending and sigmoid colon. Conclusion: A high calorie meal induces, probably via extrinsic nerves, regular myogenic contractions in the human colon at the frequency of the pacemaker cells slow waves. These myogenic patterns propagate preferentially in a retrograde direction.

ORAL-13-04

PLASTICITY OF THE SENSORIMOTOR SYSTEM FOR STANDING BALANCE IN PERSONS AFTER SEVERE STROKE

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Rehabilitation of persons after severe stroke is often limited due to their extensive impairments in sensorimotor function. Very little is known about the plasticity of the sensorimotor system in survivors of severe stroke, as most studies (using animal or human models) investigate mild to moderately-severe stroke. Previous work from our laboratory demonstrated significant improvement in muscle activation patterns in response to a unilateral arm raise perturbation in patients with mild to moderately-severe stroke after one month of inpatient rehabilitation. In addition, subjects who displayed improvement in paretic muscle activation demonstrated more recovery of functional balance compared to subjects without improvement in paretic muscle activation. Using similar methods, ten participants (58±15.1 yrs; 5 men) with severe stroke were evaluated monthly in a stroke rehabilitation unit with a functional balance scale (Berg Balance Scale) and a clinical measure of motor recovery (Chedoke McMaster Stroke Assessment). Physiological data, including weight bearing symmetry, center of pressure velocity, and electromyography (EMG) data were collected during quiet standing and during internal balance perturbations. All participants improved functionally ($p<0.05$) but physiological recovery varied, with a significant correlation between paretic EMG activation and functional balance ($r=0.59$) or motor recovery ($r=0.64$). Interestingly, EMG activation at the last testing session was correlated with admission cognitive ($r=0.53$) but not with admission motor status, as determined by the Functional Independence Measure. Our results suggest that plasticity in muscle activation associated with balance reactions is possible in some patients with severe stroke. The interplay between cognition and motor system plasticity warrants further examination. (250 words).

ORAL-13-05

FUNCTIONAL LOSS OF THE PURKINJE NEURON ENRICHED EXCITATORY AMINO ACID TRANSPORTER, EAAT4, DURING THE EARLY STAGES OF A MOUSE MODEL OF SPINO-CEREBELLAR ATAXIA, SCA1.

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Spino-cerebellar ataxia type 1(SCA1) is an incurable, autosomal dominant neurodegenerative motor disorder resulting from a CAG trinucleotide expansion of ataxin-1. In this study, we use a conditional transgenic mouse model of SCA1, where the CAG expansion is restricted to cerebellar Purkinje neurons (PNs) and where ataxia is marked beyond 12 weeks of age. Since previous studies in humans and mice report downregulation and/or mutation of excitatory amino acid transporters (EAATs) in ataxia this suggests they may be a useful target for treatment.

SCA1 mice exhibited a disruption of motor performance on the accelerating rotarod compared with controls at five weeks of age (2-way-ANOVA, $P< 0.0001$, $F_{1,20} = 68.3$). In particular, the SCA1 mice showed a similar initial motor performance to controls, but failed to improve during training.

We employed whole cell patch clamp recordings from PNs in slices prepared from the mice used in the behavioural analysis. To estimate the functional contribution of EAAT4 to PN behaviour we measured the amplitude of a pharmacologically isolated slow inward glutamatergic current triggered by synaptic stimulation. This slow current was significantly smaller in SCA1 mice compared with controls (2-way ANOVA, $P=0.0254$, $F_{1,4} =12.10$).

These results indicate the importance of the PN-enriched EAAT4 for cerebellar excitatory synaptic function and motor learning, even at the very early stages of SCA1.

(Word count 215)

ORAL-13-06

EXPRESSION OF THE DEVELOPMENTAL TRANSCRIPTION FACTOR FEZF2 IDENTIFIES A DISTINCT SUBPOPULATION OF LAYER 5 CALLOSAL PROJECTION NEURONS IN MATURE MOUSE MOTOR CORTEX

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The transcription factor encoded by Fezf2 is necessary for normal development of the cerebral cortex. However, Fezf2 continues to be expressed in the mature brain indicating that it might also be necessary for cortical function throughout life. Here, we show a unique identity of Fezf2-expressing callosal projection neurons (CPNs) in layer 5 of the mature mouse motor cortex, using a Fezf2-Gfp reporter mouse, in vivo retrograde labeling, whole-cell electrophysiology with morphology reconstruction, single-cell RT-PCR, immunocytochemistry and cluster analysis. Fezf2+ CPNs express SATB2, a protein characteristic for CPNs, but do not express CUX1 and CTIP2, proteins implicated in layer 2/3 CPNs and subcerebral projection neurons, respectively. Fezf2+ CPNs occupy layer 5A and display an apical dendritic tuft; functionally, they fire broad, adapting action potentials, and exhibit an I_h-mediated voltage sag. In contrast, CPNs without Fezf2 expression occupy layer 5B, do not display a tuft, and exhibit regular action potential firing (all $p<0.01$, Mann-Whitney, $n=32$) and smaller sag ($p<0.001$, unpaired t-test). Both groups of CPNs demonstrated distinct frequency-selective synaptic responses to corticocallosal inputs (repeated-measures ANOVA, $F(1,17) = 36.70$; Bonferroni's post-test, $p<0.01$), indicating unique contributions within the cortical microcircuitry. Our findings establish a new, distinct physiological identity of Fezf2-expressing neurons within the mature M1 CPN microcircuitry.

(228 Words)

ORAL-13-07

3D REACHES ARE ENCODED IN BODY-CENTERED, MIXED, BUT NOT HAND-CENTERED REFERENCE FRAMES IN THE MEDIAL POSTERIOR PARIETAL CORTEX OF MACAQUES

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The posterior parietal cortex (PPC) is pivotal for controlling visually guided arm reaching movements. Until few years ago, the conventional view was that caudal PPC areas use eye-centered and rostral areas hand-centered frames of reference to represent target location. However, numerous recent studies have demonstrated that multiple reference frames can be present in the same region and that large populations of neurons in several PPC areas use intermediate frames of reference. In all these studies arm movements were restricted to a single frontal plane, so the spatial representations in 3D space are unknown. We addressed this issue in the caudal PPC area V6A, where it has been shown that frontoparallel reach targets are encoded predominantly in intermediate eye- and body-centered coordinates. Single unit activity was recorded from V6A in two *Macaca fascicularis* monkeys while they performed reaches in darkness towards visual targets located at different distances and lateralities from the body. To test between body- and hand-centered representations the initial hand position was varied in depth. We found two major populations of V6A cells: a) neurons that encoded targets in intermediate body and hand-centered coordinates, and b) cells not affected at all by the initial hand position, which used body-centered representations of target location. Interestingly, we only found very few neurons that represented targets in hand-centered coordinates. Our work suggests that area specific reach representations exist in PPC and that intermediate frames of reference are widely used also to encode reaches in depth. (243 words).

ORAL-13-08

EXTRACELLULAR WILDTYPE AND MUTANT SOD1 INDUCES ER-GOLGI PATHOLOGY CHARACTERISTIC OF AMYOTROPHIC LATERAL SCLEROSIS IN NEURONAL CELLS

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Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressing neurodegenerative disorder and the majority of ALS is sporadic, where misfolding and aggregation of Cu/Zn-superoxide dismutase (SOD1) is a feature shared with familial mutant-SOD1 cases. ALS is characterized by progressive neurospatial spread of pathology among motor neurons and recently transfer of extracellular, aggregated mutant SOD1 between cells was demonstrated in culture. However there is currently no evidence that uptake of SOD1 into cells initiates neurodegenerative pathways reminiscent of ALS pathology. Similarly, whilst dysfunction to the ER-Golgi compartments is increasingly implicated in pathogenesis of both sporadic and familial ALS, it remains unclear whether misfolded, wildtype SOD1 triggers ER-Golgi dysfunction. In this study we show that both extracellular, native wildtype and mutant SOD1 are taken up by macropinocytosis into neuronal cells. Hence uptake does not depend on SOD1 mutation or misfolding. We also demonstrate that purified mutant SOD1 added exogenously to neuronal cells inhibits protein transport between the ER-Golgi apparatus, leading to Golgi fragmentation, induction of ER stress and apoptotic cell death. Furthermore, we show that extracellular, aggregated, wildtype SOD1 also induces ER-Golgi pathology similar to mutant SOD1, leading to apoptotic cell death. Hence extracellular misfolded wildtype or mutant SOD1 induce dysfunction to ER-Golgi compartments characteristic of ALS in neuronal cells, implicating extracellular SOD1 in the spread of pathology among motor neurons in both sporadic and familial ALS. (226 words).

ORAL-14-01

COLORECTAL SENSORY INPUT IS DIFFERENTIALLY PROCESSED WITHIN THE THORACOLUMBAR AND LUMBOSACRAL SPINAL CORD

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Introduction: Peripheral nerve endings of afferents innervating the colon and rectum are differentially activated by mechanical stimuli. However, it is unclear how this information is conveyed into and differentiated within the spinal cord.

Methods: Injection of different fluorescent-conjugated retrograde tracers into the lumen and sub-serosal layers of the colorectum identified afferent dorsal root ganglia (DRG) neurons and their central terminals in the spinal cord dorsal horn (DH) within the thoracolumbar (TL;T10-L1) and lumbosacral (LS;L6-S1) pathways. This was combined with pERK immunohistochemistry to identify DH neurons activated by noxious colorectal distention (CRD;80mmHg).

Results: In the TL pathway, the vast majority of traced TL-DRG neurons (86±2%) contained only sub-serosal directed tracer. Correspondingly, sub-serosal tracer was predominant in the TL-DH, with little luminally-directed tracer observed. By contrast, in the LS pathway 45±3% of traced LS-DRG neurons contained sub-serosal tracer only, with 24±1% containing luminal directed tracer only. The remained 31± 2% were dual traced. Correspondingly, in the LS-DH sub-serosal tracer was abundant in superficial laminae I, around the central canal and within the collateral tracts. Luminal tracer was most abundant within middle LS-DH regions, with a degree of co-localisation with sub-serosal tracer in laminae I. Noxious CRD activated a significant proportion of DH neurons in both the TL-DH ($P<0.0001$;N=4) and LS-DH ($P<0.001$,N=4), with the distribution pattern corresponding to that of traced terminals, with pERK neurons confined to discreet regions of the TL-DH, yet widely distributed throughout the LS-DH.

Conclusions: Colo-rectal afferent signalling is differentially conveyed and filtered between the TL and LS pathways. (250 words)

ORAL-14-02

DEVELOPMENT OF A NOVEL TRANSGENIC CGRP ALPHA REPORTER MOUSE TO FACILITATE VISUALIZATION AND DIRECT RECORDING FROM NOCICEPTIVE NERVE ENDINGS IN VISCERAL ORGANS USING CALCIUM IMAGING

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In internal (visceral) organs, no studies have recorded from the nerve endings of any spinal afferents that detect noxious stimuli. This is due largely to difficulties in identifying precisely which nerve endings belong to spinal afferents (which encode pain), as opposed to other autonomic efferent or afferent nerve endings, which also innervate the same organs. We generated a transgenic knockin mouse that expressed the red fluorescence protein, mCherry, driven by the CGRP alpha promoter, which is expressed by spinal afferent neurons. The nerve endings of CGRP expressing primary afferents could be visualized in internal organs of live tissue. On average in the colon, there were 4.3 ± 0.3 CGRP alpha-$^{mCherry\text{-}RFP}$ expressing axons identified in each myenteric ganglia (N=5). In response to circumferential stretch of the colon, calcium transients discharged simultaneously in multiple discrete varicosities along single axons of CGRP alpha-$^{mCherry\text{-}RFP}$ mice (N=5). Spritzing acid (pH 4) onto these nerve endings evoked a significant increase in the firing frequency of calcium transients in labeled nerve endings (decrease in mean interval from 7.1 to 2.9 ± 1.5 sec ($P<0.05$; N=5). In CGRP alpha-$^{mCherry\text{-}RFP}$ mice, there was a near 1:1 correlation between RFP fluorescent DRG neurons with those that were CGRP immunoreactive (N=4), confirming the transgene was expressing CGRP. This study demonstrates that CGRP alpha reporter mice can be used to visualize the location and morphology of CGRP expressing spinal afferents that encode noxious and innocuous stimuli in internal organs; and facilitates direct recording from their nerve endings.

ORAL-14-03

VISCEROSENSORY NEUROIMMUNE INTERACTIONS DISPLAY PLASTICITY WITH DISTINCT FUNCTIONAL OUTCOMES DEPENDENT UPON DISEASE STATE

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Introduction: Altered viscero-sensory neuroimmune interactions may underlie chronic abdominal pain in Irritable Bowel Syndrome (IBS) (Hughes et. al. 2012, 2013). We aimed to determine the second messengers and effector ion channels recruited by cytokines in colo-rectal afferents, and whether functional responses are altered in chronic visceral hypersensitivity (CVH). Methods: The effects that the cytokines TNF-α, IL-1β, IL-6, IL-10 and IL-2 had on pelvic colo-rectal afferents were compared between healthy and CVH (28 days post-TNBS (Hughes et. al. 2009)) mice using in vitro afferent recordings with and without antagonists for NaV1.7, TRPA1, BKCa and PI3K. mRNA expression of ion channels and cytokine receptors was determined in retrogradely-traced laser-captured colo-rectal dorsal root ganglion (DRG) neurons and compared between healthy and CVH mice. Results: In healthy mice IL-2 and IL-1β directly excited afferents, which was NaV1.7 dependent, while TNF-α and IL-6 instead caused mechano-sensitisation in a PI3K/TRPA1 dependent manner. In CVH mice colo-rectal DRG neurons had increased expression of TNFR1, IL-2R, TRPA1, NaV1.7 and BKCa but not IL-1R1, IL-10R or IL-6R. In CVH mice an increased proportion of afferents responded to IL-2 but not IL-1β. In contrast to their effects in healthy mice, TNF-α and IL-6 inhibited afferent mechanosensitivity in CVH mice, which was BKCa-dependent. However PI3K antagonism caused the TNF-α induced inhibition to switch to sensitisation, while the IL-6-mediated inhibition was PI3K independent. Conclusion: Cytokine receptor/effector ion channel coupling on colo-rectal afferents is dependent on disease state. Maladaptive coupling of cytokine receptors to effector ion channels may underlie chronic pain in IBS. (250 words)

ORAL-14-04

CONTRACTION-INDUCED FIRING OF LOW THRESHOLD STRETCH-SENSITIVE AFFERENTS IS INCREASED BY BLADDER OUTLET OBSTRUCTION

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Bladder overactivity may be secondary to increased primary afferent activation from the bladder. However, this has never been directly tested in the obstructed, overactive bladder. We used partial bladder outlet obstruction in guinea pigs, as an established way to produce bladder overactivity. Single unit extracellular recordings were made, in vitro, from fine nerve branches of pelvic afferent nerves in flat sheet bladder preparations taken from guinea pigs obstructed two weeks previously or unobstructed (control) animals. Obstructed guinea pigs showed significant increases in voiding frequency (1.12±0.19 per hour, n=11, $P<0.05$) and decreased voiding volume (1.69±0.52 ml per void, n=11, $P<0.05$) compared to sham-operated animals (0.39±0.08 per hour and 3.52±0.59 ml, n=6, respectively). In the presence of nicardipine (4uM), 7 out 10 preparations from obstructed guinea pigs showed regular contractile activity associated with bursts of firing of low threshold afferents, compared with 1 out 5 in controls. However, low threshold afferents from obstructed preparations (3.84±1 Hz for 10g, n=13) were activated less ($P<0.001$) than from controls (13.1±4.1 Hz for 10g, n=8) by an isotonic distension with a 1-20g weights. This appeared to be due to lower detrusor compliance in obstructed animals. There was no significant difference in sensitivity of low threshold afferents to the TRPA1 agonist, allyl isothiocyanate (100uM) and/or TRPV1 agonist, capsaicin (3uM) between control (n=15) and obstructed preparations (n=19). The data indicate that despite reduced compliance, there is increased firing of low threshold stretch-sensitive afferents in the obstructed bladder which is probably due to enhanced contractile activity. (247 words).

ORAL-14-05

TARGETED ANESTHESIA OF RAT NOCICEPTORS IN NORMAL AND INFLAMED BLADDER

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The quaternary lidocaine derivative QX-314 is membrane impermeable and local or systemic drug administration normally produces no anesthesia. This reflects the intracellular location of the local anesthetic binding site on neuronal voltage-gated sodium (Nav) channels. As a consequence, Nav channels are only blocked when QX-314 can access this site, which can occur in electrophysiological experiments. Recent studies show co-administration with capsaicin facilitates the passage of QX-314 through open TRPV1 channels and causes a prolonged selective anesthesia of capsaicin-sensitive nociceptors. In this study, we determined if intravesical administration of QX-314 and TRP agonists into the rat urinary bladder could provide urodynamic evidence of selective anesthesia of TRPV1 and TRPA1 nociceptors.

Intravesical drug administration was studied by continuous cystometry in adult female rats under urethane anesthesia. Drug effects were measured under normal conditions or following a 48h period of cyclophosphamide-induced experimental cystitis. QX-314 alone had no effect on baseline cystometric parameters. QX-314 co-administration with capsaicin or AITC (TRPA1 agonist) significantly reversed the urodynamic effects of prior exposure to these agonists. After cystitis, elevated urodynamic activity was significantly reduced by QX-314 alone.

We conclude that intravesical co-administration of QX-314 and TRP agonists can induce selective anesthesia of bladder nociceptors. Silencing revealed differences in the modulatory effects of TRPV1 and TRPA1 nociceptors on bladder reflex function. After cystitis, endogenous channel activation appears adequate to induce QX-314 anesthesia. This proof-of-principle study identifies a new approach to improving intravesical drug administration as a clinical therapy for common urological motor and sensory disorders and pelvic pain. (249 words)

ORAL-14-06

DUAL AND CONDITIONAL HSV-1 TRANSYNAPTIC NEURONAL TRACING OF HIGHER BRAIN SENSORY CIRCUITS ARISING FROM THE UPPER AND LOWER AIRWAYS.

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The trachea and lungs are innervated by subsets of vagal sensory neurons, yet little is known about the organisation of the brain neural circuits which receive inputs from these sensory neurons. Sensations are differentially perceived and localised from the upper versus lower airways, which may be reflected in the central neural circuits. We employed novel transneuronal tracing methods developed by our laboratory to map brain circuitries governing airway sensations. Rats (n=22) were injected with both HSV1 H129-EGFP into the lungs and HSV1 H129-tdTomato into the trachea and brains were harvested 3-7 days later. Single cell PCR was used to characterise the molecular phenotypes of tracheal and lung sensory neurons. In subsequent experiments Cre-recombinase was expressed in the nucleus of the solitary tract using an AAV vector and the trachea inoculated with a Cre-conditional HSV1 H129 which switches from green to red fluorescence in the presence of Cre (n=10). Tracheal sensory neurons expressed a somatic molecular phenotype consistent with jugular neurons, whereas lung sensory neurons were more visceral (nodose) in composition. Brain circuits receiving inputs from these sensory populations overlapped to some extent, but tracheal pathways showed preferential inputs to the mediodorsal, submedius and reticular thalamus and zona incerta whereas lung pathways projected via the parabrachial nucleus to the visceral thalamus. The upper and lower airways are innervated by different sensory neuron subtypes which provide ascending sensory inputs to distinct neural circuits in the brain. These data may explain differences in subjective sensations arising from different levels of the respiratory tree. (250 words).

ORAL-14-07

ENCODING OF FRICTION BY TACTILE MECHANORECEPTOR AFFERENTS – EFFECTS AT INITIAL CONTACT

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Aims: During object handling/manipulation, humans automatically apply forces normal to the grasped surface in proportion to the load force and scaled by the friction of the skin-object interface. Here we investigate how different types of tactile afferents may contribute to the encoding of friction at the initial contact with an object.

Methods: A 3D force controlled KUKA robotic manipulator was programmed to apply normal forces at three speeds (4, 8 and 16mm/s) to the human finger-pad. The smooth stimulation surface was tested under three frictional conditions: with lubricant, no treatment and with friction increasing agent. During stimulation, signals from single human afferents were recorded from the median nerve at wrist level.

Results: Twenty-nine afferents (12 SA-I, 2 SA-II, 12 FA-I, 3 FA-II) were recorded from 8 subjects (age 19-29 years). Kruskal-Wallis one-way analysis of variance for each afferent revealed that at 2N normal force, for 19 afferents (9 SA-I, 1 SA-II, 7 FA-I, 2 FA-II) the spike count during the initial touch was significantly different ($p<0.05$) between the three frictional conditions at least for one speed, and for 8 afferents (6 SA-I, 1 FA-I, 1 FA-I) the response was significantly different between the three frictional conditions for all three force increase speeds.

Significance: This is the first study systematically investigating afferent responses to frictional changes in the same surface. The responses of all four afferent types were affected by frictional condition; however, some influences of repeated stimulation on skin mechanics could not be fully separated.

(244 words)

ORAL-14-08

THE EFFECT OF THE THERMAL GRILL ILLUSION (TGI) ON THE RESPONSE TO INTRADERMAL CAPSAICIN IN HEALTHY MALE VOLUNTEERS

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Human experimental pain models unravel pain mechanisms and accelerate clinical drug development. We combined a novel non-nociceptive stimulus (thermal grill, TG) with a nociceptive stimulus (intradermal (i.d.) capsaicin) to further elucidate pain mechanisms and potentially investigate analgesic efficacy. The TG produces a paradoxical non-nociceptive burning sensation (TGI), whereas i.d. capsaicin activates TRPV1, a ligand gated ion channel expressed on nociceptors. The aim of this study was to compare the effect of the TGI (interlaced cool bars 20^{o}C and warm bars 40^{o}C) with standard warmth (WARM, 40^{o}C) on capsaicin-induced pain, flare, hyperalgesia and allodynia responses. 12 healthy right-hand dominant pain-free participants completed this randomised, crossover study. I.d. capsaicin (100 µg) was administered to the right volar forearm. TG or WARM stimulus was applied over the injection site pre-capsaicin and post-capsaicin for 30 s. Following TG or WARM stimulus, pain, flare, allodynia and hyperalgesia were assessed. AUCs were analysed using a two-way repeated measures ANOVA. 35% less pain was experienced from the TG compared to WARM stimulus following i.d. capsaicin (95% CI: 15%-55%). No significant differences were observed for all other outcomes. No summation of pain was observed to the TG stimulus following i.d. capsaicin. Previously, cooling and warming have shown to suppress and enhance capsaicin-induced pain respectively. The cool bars of the TG may have supressed the level of pain experienced, which is consistent with the literature. Combining the TG and i.d. capsaicin pain models is unlikely to be a useful model to investigate the efficacy of novel analgesics. (248 words).

ORAL-15-01

ALTERED FAT METABOLISM: A PATHOLOGICAL FEATURE OF DISEASE PROGRESSION IN HSOD1G93A MICE.

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While motor neuron loss and muscle atrophy are characteristic of motor neuron disease (MND), increased adiposity is correlated with slower disease progression. Increased metabolism of fat in MND may contribute to reduced fat mass and lower body mass index in MND, which is negatively associated with survival. Identification of aberrations to the key factors that maintain fat mass will provide a greater understanding of disease mechanisms. We assessed epididymal fat weight, levels of circulating non- esterified free fatty acids (NEFAs) and ketones, levels of NEFAs in skeletal muscle, the rate of lipolysis in epigonadal white fat, and the expression of genes involved in fatty acid metabolism in the hSOD1G93A mouse model of MND. We found that when compared to wild-type age-matched controls, hSOD1G93A mice failed to accumulate fat mass after the onset of disease symptoms. This was followed by subsequent increases in the expression of circulating ketones and increased NEFA in skeletal muscle, but no change in circulating NEFAs. Gene expression analysis of white adipose tissue collected from hSOD1G93A mice indicate an increase in the catabolism and mobilisation of fatty acids and oxidation of ketones. This was matched by altered lipolytic rates, relative to disease progression. Our results suggest that the inability to gain fat mass is coupled with increased breakdown and movement of fat from storage, and fat accumulation in skeletal muscle. Thus, endogenous mechanisms may promote the use of fat as an energy source in skeletal muscle in MND. (240 words)

ORAL-15-02

ALTERED NEURONAL FUNCTION IN NETWORKS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS FROM ALZHEIMER'S DISEASE PATIENTS

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Induced pluripotent stem cells (iPSCs) from patients is facilitating research into neurodegenerative disease pathogenesis. These cells are created by 'reprogramming' adult somatic cells to a pluripotent state. The iPSCs can be directed to differentiate into neurons through incubation with specific concentrations of growth factors, mimicking neuronal differentiation in vivo. The neuronal iPSC derived models exhibit phenotypes synonymous with their in vivo counterparts and provide a means to interrogate sporadic diseases, including Alzheimer's disease. Using a combination of advanced mass spectrometry, chromatography and imaging techniques we have identified key changes in lipids and proteins in cells from Alzheimer's disease patients. By promoting the formation of neuronal networks from patient iPSCs we have further interrogated the mechanisms by which these changes alter neuronal function in vitro. Using a variety of techniques we have tracked these changes during differentiation from pluripotent cells into neurons. These changes lead to extensive functional effects on neuronal signalling events. Our data establishes lipids as key players in differentiation and identifies major changes in specific lipid species in Alzheimer's disease patient cells. (Word count: 174).

ORAL-15-03

ERBB4 PROTEIN AND MRNA EXPRESSION IN NEUREGULIN 1 TRANSMEMBRANE DOMAIN HETEROZYGOUS MOUSE VARIES ACCORDING TO AGE AND SEX

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Neuregulin 1 and its receptor, ErbB4, have been implicated with schizophrenia at both the genetic and molecular level. Sex specific schizophrenia-like behavioural phenotypes have been observed in the neuregulin 1 transmembrane domain heterozygous mutant (Nrg1 TM HET) mouse however the impact of sex, age and Nrg1 TM mutation on ErbB4 levels have not been elucidated. To test the impact of these factors on ErbB4 levels, we analysed the protein and mRNA expression of ErbB4 in the hippocampus. ErbB4 protein levels at postnatal day (PND) 35 in Nrg1 TM HET and wild type-like (WT), male and female mice were analysed using immunoblotting. ErbB4 mRNA expression levels were measured in male and female Nrg1 TM HET and WT-like mice at ages PND1, 7, 14, 49 and 161 via in situ hybridisation. Immunoblotting revealed an elevation of ErbB4 protein in females compared to males in WT-like mice ($F(1,17)=5.381$, $p=0.033$, $n=5$-6), but no difference between sexes in Nrg1 TM HET mice. Similarly, PND49 female WT-like mice had elevated ErbB4 mRNA levels compared to males ($F(1,90)=7.382$, $p=0.008$, $n=4$-7), but no difference between sexes in PND49 Nrg1 TM HET mice were detected. PND1, 7, 14 and 49 female mice show elevated levels of ErbB4 mRNA compared to males at these ages. Overall, ErbB4 mRNA is downregulated as age increases ($p<0.0001$). These results suggest that ErbB4 protein and mRNA expression is sensitive to sex and age and may play a role in mediating sex specific schizophrenia-like phenotypic behaviours in this Nrg1 TM HET mouse model of schizophrenia (250 words).

ORAL-15-04

CHARACTERISATION OF RETINAL GANGLION CELL SURVIVAL AND AXON REGENERATION FOLLOWING PULSED MAGNETIC FIELD STIMULATION IN A MOUSE OPTIC NERVE CRUSH MODEL.

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Pulsed Magnetic Fields (PMF), a non-invasive form of brain stimulation, have been shown to induce structural and functional brain plasticity, including short distance axonal sprouting in a cerebellar lesion model. However, the potential for PMFs to promote long distance axonal regeneration following neurotrauma has not been investigated. This study examined whether PMF can increase RGC survival and induce axon regeneration in an optic nerve crush (ONC) neurotrauma model.

Three month old C57/Bl/6J mice received a unilateral intraorbital optic nerve crush. Mice received 10 minutes of sham (n=8) or PMF (n=9) daily stimulation for 14 days to the operated eye. Immunohistochemistry for Tuj1 and Gap43 was used to assess RGC survival (retina) and axon regeneration (optic nerve) respectively.

A separate group of ONC mice received sham (n=5) or PMF (n=5) for BDNF ELISA analysis to quantify changes in BDNF in the retina and optic nerve.

Preliminary optic nerve results show no Gap43 positive labelling for sham (n=3) or for PMF (n=3) stimulation. Mean crush site widths (relative to total optic nerve width) for sham and PMF are 67.8%±16.7% and 59.5%±9.7% respectively. There was no significant difference in BDNF in the retina or optic nerves between PMF and SHAM ($p>0.05$). Stereological analysis of RGC survival is currently in progress.

Whilst PMF has been shown to induce short distance sprouting in some models of neurotrauma, preliminary results suggest PMF does not induce regeneration following an optic nerve crush. These results will help define the benefits and limitations of PMF treatment following neurotrauma. (249 words)

ORAL-15-05

INHIBITORY LOSS OR DYSFUNCTION: A PRIMARY MECHANISM IN AMYOTROPHIC LATERAL SCLEROSIS ?

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In ALS, increased excitability of circuitry precedes motor neuron degeneration, suggesting that ALS results from disturbances in regulation of cell excitability. However, the mechanism of presymptomatic excitability remains unknown. We examined the motor and somatosensory cortex of $SOD1^{G93A}$, $TDP43^{A315T}$ and non-transgenic mice for expression of interneuron-specific calcium binding and neuropeptide protein markers. Studies were also performed in human ALS and control brain tissue, investigating the presence of pathological changes within interneuron populations. Cortical tissue from presymptomatic (8 week) and end-stage (20 week) $SOD1^{G93A}$ and ALS human cortex were serially sectioned (40um), alongside age-matched controls (n=4 for all animals, n=6 for human cases), and immunohistochemically labelled with antibodies against calretinin (CR), parvalbumin (PV), somatostatin (SOM), Neuropeptide Y (NPY) and Vasoactive Intestinal protein (VIP) prior to confocal analysis. Analysis of presymptomatic $SOD1^{G93A}$ animals found CR^+ interneurons to be unchanged (22.218 ± 3.607) relative to controls (27.660 ± 3.404). Analysis of end-stage $SOD1^{G93A}$ cortical regions, however, found the number of CR^+ interneurons in the supragranular motor cortex lamina (I-IV) was significantly decreased ($P<0.05$) by 37% (12.53 ± 2.137 SEM), as compared with wild type (19.59 ± 2.452) (Two-way ANOVA, multiple comparisons Bonferroni Test). Analysis of end-stage $SOD1^{G93A}$ NPY^+ interneuron numbers in the infragranular motor cortex (V-VI) identified a significant increase ($P<0.05$) by 40% (11 ± 1.493), as compared with wild type (6.592 ± 0.879). These findings demonstrate pathological alterations to inhibition in the end-stage $SOD1^{G93A}$ model of ALS, specifically implicating CR and NPY interneuron populations in cortical dysfunction.

ORAL-15-06

NEDD4-FAMILY INTERACTING PROTEIN 1 (NDFIP1) IS INVOLVED IN THE DNA DAMAGE RESPONSE VIA THE ATAXIA TELANGIECTASIA MUTATED (ATM) MECHANISM

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DNA damage response (DDR) is a crucial process for activating the DNA repair process in neurons following brain injury. BAAT1 (BRCA1-associated ATM activator 1) is known to regulate the phosphorylation of ATM, a major protein kinase required for activating DDR. In this study, we investigated the role of NDFIP1 in regulating BAAT1 and ATM following cell stress. NDFIP1 is an activator of Nedd4 ubiquitin ligase and has previously been shown by our group to mediate the neuroprotective response following brain injury and ischemia. We found that expression of NDFIP1 was associated with increased phosphorylation of ATM in a DNA damage model. NDFIP1 was also found to bind and increased the ubiquitination of BAAT1 for nuclear transport. Following brain injury, surviving neurons with increased NDFIP1 also exhibited translocation of BAAT1 to the nucleus where it is known to bind ATM. Thus, NDFIP1 can increase the survival of neurons following injury by activating DNA repair mechanisms via the ATM pathway. This is in addition to other mechanisms of neuroprotection by NDFIP1, including restricting metal entry and increasing phospho-AKT by reducing cytoplasmic PTEN.

ORAL-15-07

INVESTIGATING THE ROLES OF EPH/EPHRIN AFTER FOCAL ISCHEMIA IN THE NONHUMAN PRIMATE VISUAL CORTEX

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Australian Regenerative Medicine Institute, Monash University, 3800 VIC, Australia The mammalian receptor tyrosine (Eph)-interacting ligand (ephrin)-A2 plays crucial roles in axonal guidance and cell migration during early CNS development, but little is known about its role postnatally and following brain injury. Recent studies in human suggest that ephrin-A2 reverse signaling promotes cell-survival in the presence of cytotoxicity. Therefore, we aim to identify whether ephrin-A2 confers similar roles in the acute and sub-acute periods following a focal ischemia (FI) of infant and adult marmoset monkey V1 and identify a potential mechanism of neuroprotection.

FI was induced in infant (P14; n=4) and adult marmosets (n=5) through chemically- induced (endothelin-1) occlusion of the calcarine artery supplying operculum V1 territory. Brains were harvested at 1 and 21 days-post-ischemia (DPI). Ephrin-A2 was upregulated on interneurons by 1DPI and sustained in the peri-infarct area of neonates but not to the same extent in adults. Subcellular examination revealed changes in ephrin-A2 localisation, from a diffuse cytoplasmic distribution to a more aggregated morphology around the cellular periphery, indicating membrane-bound clustering. No change in the EphA4 receptor expression was detected at 1DPI but was upregulated on reactive astrocytes proximal to the infarct by 21DPI. Cell-cell contact between ephrin- A2+ neuronal soma by EphA4+ reactive astrocyte processes was detected at 21DPI but not 1DPI.

Our results demonstrate that neuronal ephrin-A2 signaling was initiated acutely after FI and sustained sub-acutely in neonates only, implicating a role for ephrinA2 in neuroprotection in response to CNS injury. We propose that ephrinA2 reverse signaling through astrocytic EphA4 receptor promotes neuronal survival in primates after FI. (250 words)

ORAL-15-08

MARKERS OF INFLAMMATION AND STRESS DISTINGUISH SUBSETS OF INDIVIDUALS WITH SCHIZOPHRENIA AND BIPOLAR DISORDER

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Schizophrenia and bipolar disorder share a number of common features, both symptomatically and biologically. Abnormalities in the neuroimmune and the stress-signaling pathways have been previously identified in the post-mortem brains of individuals with both diseases. However, a possible relationship between abnormalities in stress and neuroimmune signaling within the cortex of people with psychotic illness has not been defined. To test the hypothesis that combined alterations in brain stress responsiveness and neuroimmune/inflammatory status are characteristic of some individuals suffering from major mental illness we examined gene expression in the Stanley Array Cohort of 35 schizophrenia, 34 bipolar disorder and 35 control individuals. We used levels of 8 inflammatory related transcripts, of which SERPINA3 was significantly elevated in individuals with schizophrenia ($F(2,88)=4.137$, $p<0.05$), and 12 glucocorticoid receptor (GR) signaling (stress) pathway transcripts previously examined to identify two clusters of individuals with high (n=32) and low (n=68) inflammation/stress transcript expression. The high inflammation/stress transcript group has a significantly greater number of individuals with schizophrenia (n=15), and showed a trend towards having more bipolar disorder individuals (n=11), when compared to controls (n=6). Using these groups, we tested which microarray assessed gene expression changes may be associated with high immune/stress signaling pathways using ingenuity analysis and found that a more extended network involving immune function, growth factors, inhibitory signaling and cell death also distinguished these groups. This implies that not only is the heterogeneity in schizophrenia partially explained by inflammation, stress and inflammation/stress interactions, but similar heterogeneous clusters may also occur in bipolar disorder. (249 words).

ORAL-16-01

EARLY LIFE DIET PROGRAMS RESPONSES TO AN IMMUNE CHALLENGE

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Obesity is characterized by a chronic low-grade inflammation. At the level of the hypothalamus, this inflammation can lead to a disruption of the pathways regulating feeding and metabolism and a resistance to satiety signalling from leptin and insulin. Although hypothalamic inflammation can be influenced by diet at any stage of life, the early life nutritional environment is likely to be particularly important because of its potential to influence how central immune cells mature. We have seen rats suckled in small litters (of 4; SL) develop an overweight phenotype, compared with those suckled in control litters (of 12; CL), that is evident by as early as postnatal day (P) 7 and persists into adulthood. These SL rats also have exacerbated neuroimmune responses to an immune challenge. We hypothesized this exacerbated pro-inflammatory response may be due to alterations in the normal maturation of microglia within the young brains of neonatally overfed rats, by encouraging the microglia to remain in a 'primed' or sensitized state that can contribute to a basal pro-inflammatory profile. We examined inflammatory profiles, including microglial morphology, in rats suckled in CL or SL at P7 and P14 and found neonatally overfed rats have more microglia in the paraventricular nucleus, and other regions of the hypothalamus, accompanied by changes in the pro-inflammatory profile. These findings suggest neonatal overfeeding can alter microglial maturation, potentially contributing to exacerbated central responses to an immune challenge. (232 words)

ORAL-16-02

THE IMPACT OF TOLL-LIKE RECEPTOR 4 ON THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS

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Introduction:
Toll-like receptor 4 (TLR4) is part of the innate immune system, and TLR4 activation triggers the Hypothalamus-Pituitary-Adrenal (HPA) axis. Genetic knock out of TLR4 results in a hyporesponsive HPA axis to immune stress, but little is known about TLR4 involvement in the psychological stress response even though a dysregulation of both immune and HPA systems is evident in multiple psychological disorders. The current study aims to investigate the role TLR4 plays in HPA modulation by characterising strain differences between TLR4 genetic knockout (KO) and wild type Balb/c (WT) mice in their HPA anatomy, behavioural and HPA responses to forced swim stress and direct adrenal activation to investigate the role TLR4 plays in HPA modulation.

Results:
KO mice display smaller adrenal cortex size, and a corresponding lower level of baseline and post stress circulating corticosterone concentration, but higher adrenocorticotrophic hormone (ACTH) and corticosteroid binding globulin (CBG) expression. Higher levels of CBG expression were also evident in the hippocampus but no strain difference was found in the hypothalamus. However, a maximal dose of ACTH to directly activate the adrenal glands revealed that KO and WT adrenals were able to achieve the similar corticosterone output.

Conclusion:
Genetic KO of TLR4 influences HPA activity on a fundamental level, but this difference is likely to lie in the higher structures rather than in adrenal function. (221 words)

ORAL-16-03

MICROGLIAL ACTIVATION IN THE HYPOTHALAMUS, MIDBRAIN AND MEDULLA FOLLOWING MYOCARDIAL INFARCTION

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Following myocardial infarction, microglia, the immune cells in the central nervous system, become activated in the hypothalamic paraventricular nucleus (PVN) suggesting inflammation occurs in this nucleus. There is little is known about effects on other brain nuclei. In the present study, we investigated whether the rostral ventrolateral medulla (RVLM), the nucleus tractus solitarius (NTS) and the periaqueductal gray (PAG), regions known to have important cardiovascular regulatory functions, also show increased microglial activation. We also investigated the effect of administering the anti-inflammatory drug, minocycline, into the brain ventricles on microglial activation and heart function. Sprague Dawley rats were infused with either saline (0.09% NaCl) or minocycline (172ng/ml, 0.3 ul/hr) into the lateral ventricle of the brain. The animals then underwent either a myocardial infarction (MI) or sham procedure. Cardiac function, was determined by echocardiography 12 weeks post MI and then the animals were killed and the brains processed immunohistochemically to detect activated microglia using the presence of the marker protein CD11b. Compared to controls (N=8), MI elicited a significant increase in the proportion of activated microglia in the PVN, RVLM, NTS and PAG, (N=3)($P<0.001$). Minocycline significantly attenuated the response by at least 50% in each brain region (N=5). Cardiac function was significantly reduced by 55 % following MI but this was not ameliorated by minocycline. The results suggest that following MI, inflammation occurs in brain nuclei that play key roles in cardiovascular regulation and that attenuation of this inflammation may not be sufficient to improve cardiac function. (246 words)

ORAL-16-04

THE CARDIOVASCULAR ACTIONS OF FRACTALKINE/CX3CL1 IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ARE ATTENUATED IN HEART FAILURE RATS.

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The paraventricular nucleus (PVN) of the hypothalamus plays an important role in the regulation of sympathetic nerve activity (SNA), which is significantly elevated in chronic heart failure (CHF). Fractalkine (FKN) and its cognate receptor, CX3CR1, are constitutively expressed in the central nervous system but its role and physiological significance are not well known. The aims of this study were to determine whether FKN plays a cardiovascular role within the PVN and to investigate how the actions of FKN might be altered in CHF. Here we show that both FKN and CX3CR1 are expressed on rat PVN neurons. Unilateral PVN microinjections of FKN in pentobarbitone (60mg/kg ip) anaesthetised rats elicited a significant dose-related decrease in blood pressure (1.0nmol, -5±3 mmHg; 2.5nmol, 13±2; 5.0nmol, 22±3 mmHg; 7.5nmol –32±3 mmHg) and a concomitant increase in heart rate (1.0nmol, 6±3 bpm; 2.5nmol, 11±3; 5nmol, 18±4 bpm; 7.5nmol 27±5 bpm). Additionally, we found a significant increase in CX3CR1 mRNA and protein expression, as determined by quantitative RT-PCR and Western blot analysis respectively, in PVN of rats with CHF compared to sham controls. The blood pressure effects of FKN (2.5 nmol in 50 nl) were significantly attenuated in rats with CHF (ΔMAP -6±3 mmHg) compared to sham-operated control (ΔMAP -16±6 mmHg) rats. These data suggest that FKN and its receptor, CX3CR1 modulate cardiovascular function at the level of the PVN and that its actions within this nucleus are attenuated in heart failure. (238 words).

ORAL-16-05

THE INVOLVEMENT OF MIDBRAIN REGIONS IN COUGH HYPERSENSITIVITE PATIENTS

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Cough hypersensitivity (CH) disorder is characterised by lowered cough reflex thresholds and persistent sensations of the urge-to-cough (UTC), suggesting dysfunction of components of the cough neural network. The purpose of this project was to investigate regional brain responses during inhalation of a tussive substance (capsaicin) in participants with CH compared to age and sex matched healthy controls using functional magnetic resonance imaging. CH participants (n=16) and healthy controls (n=16) inhaled increasing doses of capsaicin to determine thresholds for UTC, cough and maximum suppressible dose (C_u, C_2 and S_{max} respectively). Blood oxygen level-dependent (BOLD) images were collected during capsaicin-evoked UTC challenges at S_{max} for all participants to allow between-group comparisons during like-subjective experience. Controls also underwent UTC challenges during inhalation at the capsaicin dose of their matched CH participant to allow like-stimulus intensity comparisons. Results showed significantly decreased C_u, C_2 and S_{max} in CH compared with control participants ($t(34)=2.72$, $p=0.01$; $t(34)=2.64$, $p<0.05$, $t(31)=2.17$, $p<0.05$ respectively). Between-group differences were also seen for BOLD signal changes in midbrain regions (nucleus cuneiformis and periaqueductal gray) during UTC challenges in both like-subjective experience and like-stimulus intensity comparisons. The CH group showed graduated BOLD responses to two doses of capsaicin as well as responses to saline (normally an innocuous stimulus). Percentage BOLD signal in the midbrain was negatively correlated with C_u of CH participants demonstrating increased activation with increased airway sensitivity ($R^2=0.29$). These results suggest that CH may involve central sensitisation to airway inputs that could possibly be mediated by altered responses in midbrain regions. (249 words)

ORAL-16-06

PRESYNAPTIC AND POSTSYNAPTIC Y RECEPTORS ACT DIFFERENTIALLY TO MODULATE PULSATILE GROWTH HORMONE SECRETION IN THE MOUSE

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The neuropeptide Y (NPY) system in the brain plays an important role in regulating food intake and energy expenditure. Different biological actions of NPY are specifically assigned to NPY receptor subtypes. While early studies in rodents demonstrated an inhibitory role of NPY on GH axis, they provided limited insights into the mechanistic neuronal interconnectivity and the receptor involvement between the NPY and GH regulating system.
Using genetically modified mice (germ-line NPY, Y1 and Y2 receptor knockout), pulsatile GH secretion was assessed under both fed and fasting conditions. We found that deletion of NPY maintained pulsatile GH secretion in mice following 6 hours of food withdrawal. The recovery of GH secretion as observed in NPY knockout mice was associated with a reduction in *SRIF* mRNA expression. Furthermore, we demonstrated that the postsynaptic Y1 receptor contributed to the suppression of GH secretion during short-term starvation whereas the presynaptic Y2 receptor was responsible for maintenance of normal GH output under long-term fed conditions. Data demonstrate the integration of neuronal mechanisms that modulate GH release to food intake and may provide essential information to address the different role of Y receptors in regulating anabolic GH profiles under distinctive energetic conditions. (196 words).

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ORAL-16-07

TRANSGENIC RATS WITH ATAXIN3-MEDIATED DESTRUCTION OF OREXIN NEURONS SHOW A REDUCED BROWN ADIPOSE TISSUE THERMOGENIC RESPONSE TO CONFRONTATION WITH A CAGED INTRUDER RAT

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Hypothalamic orexin-synthesizing neurons influence behavioural and physiological interactions with the environment. Mice with ataxin3-mediated (Orx/Atx3) destruction of orexin neurons have reduced restraint-induced temperature increases (Zhang et al 2010). In transgenic Orx/Atx3 rats, body temperature has been reported (Schwimmer et al, 2010), but so far there are no reports of brown adipose tissue (BAT) thermogenesis during emotional hyperthermia. We now investigate BAT and body temperature changes induced in Orx/Atx3 rats by sudden confrontation with a Sprague-Dawley rat confined to a small cage. Transgenic or wildtype male rats (300-450g) anesthetized with inhaled isoflurane (2% in oxygen) were instrumented with chronically implanted thermistors to measure BAT (interscapular) and body (mediastinal) temperatures, in accord with the Flinders University Animal Welfare Committee. After one-week recovery the unrestrained rats were housed singly in a quiet closed temperature-controlled (24-26°C) cage. A normal Sprague-Dawley rat confined to a smaller cage (intruder) was suddenly introduced into the cage of the resident rat. Pre-intruder BAT and body temperatures were compared with temperatures recorded 26-30 min after the introduction of the intruder. The intruder-induced increases in BAT and body temperatures in transgenic rats (0.51±0.14C° and 0.39±0.17C° respectively, n=6) were less than the corresponding values in wildtype rats (1.42±0.19C°, $p<0.01$, and 0.84±0.0.10C°, $p<0.05$ respectively, n=9). For both wildtype and transgenic rats, increases in BAT temperature were greater ($p<0.01$) than increases in body temperature. Our results demonstrate that the orexin-synthesizing neurons participate in regulating the BAT thermogenesis that contributes to emotional hyperthermia. (241 words).

ORAL-16-08

DIFFERENT FOREBRAIN REPRESENTATION OF THERMAL AND PSYCHOGENIC SWEATING IN HUMANS

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We recently showed in humans that sweating evoked by thermal and mental stress activates the same brainstem regions. In the forebrain, others have shown that the dorsal mid cingulate cortex is activated with sweating due to mental stress and exercise, but the regions associated with thermal sweating are unclear. Here we sought to: 1. test whether thermal sweating activates this same cingulate cortex region, 2. look for functional connectivity between forebrain regions and the presumptive common output pathway in the brainstem. Two groups of 11 participants were either subject to whole body heating or undertook a mental task during the acquisition of functional brain images using a magnetic resonance imaging scanner. Concurrently recorded galvanic skin responses were used as regressors in analyses of brain images to identify forebrain regions with signal increases associated with sweating events. Additional regressors based on signals extracted from the putative output nucleus in the rostral medulla were used to test for functional connectivity between this region and other parts of the brain. Sweating during mental stress was confirmed to cause activation in the dorsal mid cingulate cortex (BA24, $p<0.05_{corrected}$), and this region showed significant functional connectivity to the putative medullary output nucleus. Sweating activation and functional connectivity during thermal stimulation were found in a distinct cingulate cortical region - the pregenual region (BA32, $p<0.05_{corrected}$). These findings suggest a functional topography within the cingulate cortex, whereby distinct regions may drive thermal and mental sweating via their connections to the common brainstem output pathway.

ORAL-17-01

CHARACTERISATION OF THE STABILITY AND BIO-FUNCTIONALITY OF TETHERED PROTEINS ON BIOENGINEERED SCAFFOLDS: IMPLICATIONS FOR NEURAL REPAIR

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Various engineering applications have been utilised to deliver molecules and compounds in both innate and biological settings. In the context of biological applications, the timely delivery of molecules can be critical for cellular and organ function. As such, previous studies have demonstrated the superior benefit of long- term protein delivery, by way of protein tethering onto bioengineered scaffolds, compared to conventional delivery of soluble protein in vitro and in vivo. Despite such benefits little knowledge exists regarding the stability, release kinetics and functionality of these proteins over time. As way of example, here we examined the stability, degradation and function of a protein, glial derived neurotrophic factor (GDNF), which is known to influence neuronal survival, differentiation and neurite morphogenesis. Enzyme-linked immunosorbent assays revealed that GDNF, covalently tethered onto polycaprolactone (PCL) electrospun nanofibrous scaffolds, remained present on the scaffold surface for 120 days, with no evidence of protein leeching or degradation. The tethered GDNF protein remained functional and capable of activating downstream signalling cascades for up to 3days, as revealed by its capacity to phosphorylate intracellular Erk in a neural cell line. Furthermore, immobilisation of GDNF protein promoted cell survival and differentiation in culture at both 3 and 7 days, further validating prolonged functionality of the protein, well beyond the minutes to hours timeframe observed for soluble proteins under the same culture conditions. This study provides important evidence of the stability and functionality kinetics of tethered molecules.

ORAL-17-02

SELF-ASSEMBLING PEPTIDE HYDROGELS FOR REPAIR OF NEURAL CIRCUITRY

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Novel biomaterials for regeneration of damaged central nervous system (CNS) have emerged as important tools for improving cell replacement therapy (CRT) [1]. These biomaterials are designed to provide physical and biochemical cues to cells, similar to that offered by the extracellular matrix (ECM), in order to promote cell survival, proliferation and direct differentiation *in vivo*. One class of biomaterials includes self-assembling peptides (SAPs). Using a "bottom-up" approach, SAPs exploit simple, non-covalent interactions to form highly complex nanofibrous architectures, important for physical cellular support, and can additionally incorporate bioactive signals[2].

Fmoc-based SAPs have the capacity for self-assembly under physiological conditions via π-β interactions. The Fmoc groups form π-bonds to form the nanofibres, with the attached peptides stabilising the structure through formation of β-sheets[3]. The overall material presents as an implantable hydrogel. Recently, we have incorporated a laminin sequence, IKVAV, into this Fmoc-SAP system[4]. As a result of the π-β interactions, the peptide sequence is presented on the outer surface of the nanofibres at high density for access to cells.

The resulting material is a sophisticated ECM mimic in terms of physical structure (the nanofibrous architecture) and biochemical cues (presentation of IKVAV at high-density). In combination with CRT, Fmoc-SAPs can provide the required support for implanted cells in models such as Parkinson's disease, potentially improving cell survival, regeneration of damaged tissue and host neural circuitry repair. (224 words)

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ORAL-17-03

CLOSE-FIELD ELECTROPORATION VIA THE COCHLEAR IMPLANT ENHANCES THE BIONIC INTERFACE

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The cochlear implant (CI) is a remarkable device that has provided great benefit to the lives of hundreds of thousands of individuals suffering profound hearing impairment. A major limitation of the CI is the quality of sound perception. It is thought that this could be improved if finer neural recruitment could be provided. Previously it has been demonstrated that the introduction of neurotrophins, such as Brain-Derived Neurotrophic Factor (BDNF), into the cochlea can enhance survival of the spiral ganglion neurons (SGNs) in a deafened guinea pig model. We have developed a novel form of gene delivery to transform mesenchymal cells lining the cochlear compartments that we have termed Close-Field Microarray-based Electroporation (CFMAE). This technique utilises the CI microarray interface to deliver focused electric fields within the cochlea capable of transforming cells via electroporation. Using this method we induced cells to secrete BDNF, improved SGN survival and enhanced the bionic interface. (150 words)

ORAL-17-04

CORTICAL NEUROPATHOLOGY FOLLOWING IMPLANTATION OF WIRELESS MULTIPLE ELECTRODE ARRAY FOR BIONIC EYE PROSTHESIS

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We are developing a cortical implantable prosthetic device for restoration of some form of vision in the blind. Here we report the acute tissue injury and long term biocompatibility in sheep of a putative cortical implant.

Custom made electrode arrays (MiniFab, Melbourne). consisting of a tile base and 45 parylene-coated platinum/iridium electrodes were implanted into the parietal lobe. Animals were allowed to recover for 2 weeks, 3 or 6 months (one animal per timepoint) before brains were retrieved. NeuN, GFAP, CD68 and MAP2 immunohistochemistry were used to investigate neuronal survival, astrogliosis, macrophage infiltration and dendritic integrity.

Post-mortem examination showed that at all time-points, the brains appeared normal without visible lesion, hematoma or hypotrophy around array implantation sites. The tiles were stable on the cortical surface with granulation encapsulation. Tissue damage was restricted only to areas close to the electrode insertion tracks. Astrogliosis and microglia were increased around the electrodes at 2 weeks, then decreased at 3 months and remained unchanged at 6 months. Across all three time points there was a significant reduction in neurons and dendrites within the first 50µm from the electrode, returning to normal levels by 100µm from the electrode edge ($p<0.05$).

In summary, implantation of penetrating electrode arrays into the sheep brain caused minimal damage. Inflammation was short lasting and stabilised by 3 months after implantation. Moderate neuronal cell loss was found, and only close to the electrode track. This electrode material appears to be biocompatible for at least 6 months after implantation.

ORAL-17-05

TESTING DIFFERENT MODELS OF THE ORGANISATION OF THE DORSAL EXTRASTRIATE CORTEX USING MULTIELECTRODE ARRAYS

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Given the imperative of sampling cells with receptive fields in different parts of visual space, most of what we know about the detailed structure of visuotopic maps comes from qualitative studies in which single electrodes were moved to cover different portions of an area. Here, we demonstrate the feasibility of simultaneous, stable quantitative mapping of the receptive fields of neurons across large expanses of cortex in marmoset monkeys, using 96-channel multielectrode arrays. The marmoset is an ideal model for this type of work, given that most areas are accessible on the surface of the brain. The animals were anaesthetized with sufentanil (8 µg/kg/h) and N_2O (70% in oxygen), and the eyes were focused on a computer screen covering more than 40° of visual field. We investigated the long-standing question of whether the dorsomedial [DM] and second [V2] visual areas are adjacent, or are separated by a distinct third visual area [V3]. Our results provide conclusive evidence in support of the former model: The lateral portion of DM comprises a representation of the upper visual field that is directly adjacent to V2. More medially, DM also includes a lower quadrant representation that, when considered in isolation, could be mistaken as V3. The sizes of receptive fields in the upper and lower quadrant representations of DM were identical for a given eccentricity, and neurons responded to visual stimulation with similar latencies. The location of DM suggests that it corresponds to the sixth visual area [V6] described in the macaque and human brain.

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ORAL-17-06

A PREDICTIVE MODEL OF SURFACE LIGHTNESS PERCEPTION THROUGH TRANSPARENT LAYERS.

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A key unsolved problem in vision science is to develop a quantitative theory of surface lightness perception through transparent layers, such as those associated with shadows, highlights, smoke, fog and other atmospheric media. Here, we show how surface lightness perception through such transparent layers can be generically understood in terms of a new computational theory of surface perception that has been shown to quantitatively account for a wide range of perceptual data on surface lightness, transparency and gloss perception [Vladusich T., Vision Research 69, 2012: 49-63; and J. Vision 13:1-21, 2013]. The theory unifies many extant theoretical concepts in terms of a simple mathematical framework with sophisticated computational properties. Using computer simulations based on our mathematical model, we show that the theory can quantitatively predict perceptual data from two well-known visual perception experiments on surface lightness perception through transparent layers. A key feature of the theory that enables its predictive power is that it discards the prevailing assumption that the dimensional structure of the brain's representation of the physical world corresponds to the dimensional structure of the physical world itself. The theory instead posits that the form of the representation is determined by the computational organisation of the brain, and takes the form of different gray-scale "gamut lines" representing perceived illumination/transparency/gloss levels in a blackness-whiteness vector space. Such an approach enables the theory to suggest how stimulus- and task-driven constraints combine to control processes underlying vector computation. (237 words).

ORAL-17-07

RELATION OF KONIOCELLULAR PATHWAY ACTIVITY TO LOW FREQUENCY (DELTA) ELECTROENCEPHALOGRAM POWER IN ANAESTHETISED MARMOSETS.

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Purpose: We previously reported that some neurons in the intercalated (koniocellular, KC) layers of the lateral geniculate nucleus (LGN) show high variability in maintained discharge rate that is inversely correlated to low frequency power in the electroencephalogram (EEG) in the primary visual cortex (V1) [1]. Our purpose here is to find the source of this variability, specifically its time-relation to low frequency EEG.
Methods: Extracellular spike activity of LGN neurons (n=114) and local field potential from V1 were recorded in sufentanil-anaesthetised marmosets (*Callithrix jacchus,* n=12). The visual stimulus was a uniform grey field ~15 degrees square at 50 Cd / m^2. The phase of KC neuron spikes relative to cortical delta oscillations was calculated by filtered Hilbert transform of the V1 data. Data are expressed as mean±SD.
Results: Koniocellular neurons showed significantly variability in maintained discharge rate SD (5.18±0.62, n=44) compared to Parvocellular (2.72±0.20, n=45) and Magnocellular (3.13±0.34, n=25) neurons. Sixteen out of nineteen KC neurons with SD > 5 show inverse correlation with V1 delta power. LGN spikes occur during any phase of delta frequency oscillation in V1 with equal probability irrespective of delta power.
Conclusion: These results indicate that KC neurons have higher firing rate variability than PC and MC neurons inversely correlated to V1 delta power, however LGN spikes are not phase locked to V1 delta frequency oscillation indicating LGN and V1 are not tightly coupled as an oscillating system.

[1] Cheong S.K. et. al., (2011) *PNAS* 35, 14659-14663. (242 words)

ORAL-17-08

DISTRIBUTED CODING OF VISUAL SPACE IN MARMOSET AREA MT

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Purpose: Area MT of the primate cortex forms part of the 'where' stream of visual processing, important in establishing the motion and position of objects. But the receptive fields of individual neurons in area MT are thought to be too large to provide a precise signal for spatial vision. We investigate whether overlapping receptive fields in area MT leads to a population code capable of fine spatial precision.

Method: The population response of area MT was measured in Sufentanil-anaesthetised marmosets (Callithrix jacchus) (n=3). Recordings were made using a 96 channel electrode array (Utah) positioned in area MT in one hemisphere of the animal. The visual stimulus was a white disc moving along linear trajectories at 20°/s. Multiunit activity was analysed in time bins of 0.05 s.

Results: Receptive fields at each electrode were extensive and highly overlapping, such that each position in visual space projected onto a different subpopulation of the recorded neurons, forming a distributed network coding of spatial position. Using linear support vector machines, we found that this distributed network was capable of discriminating the 1° displacement between neighbouring sample points. This capacity was supported by neurons whose receptive fields flanked the target. Spatial discriminability improved further 20-30% when correlations between neurons were taken into account.

Conclusion: These results show that distributed encoding allows spatial precision even in the absence of small receptive fields. We speculate that distributed coding also extends to other visual properties of the neural response including stimulus orientation, motion direction, and motion speed.

(Word count: 249)