POSTERS

POS-MON-001

IMPORTANT ROLE FOR TEN-M2 IN THE FORMATION OF FUNCTIONAL BINOCULAR CIRCUITS IN THE MOUSE VISUAL SYSTEM

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Increasing evidence suggests that multiple Teneurin (Ten-m) molecules have important, complementary roles in the organisation of binocular circuits. Previous experiments have indicated a specific role for Ten-m2 in regulating the size and location of ipsilateral retinal projections, with Ten-m2 KO mice having fewer ipsilateral projections arising from ventral retina projecting to their topographically-corresponding target region (rostral) within the dorsal lateral geniculate nucleus (dLGN). Here, we look at the impact that these changes may have on visual function and behaviour of animals lacking Ten-m2. Immunostaining for the immediate-early gene, c-fos, revealed that the area of primary visual cortex (V1) driven by ipsilateral inputs was significantly reduced ($p<0.05$, multivariate ANOVA) in Ten-m2 KO mice (n=6), compared to WTs (n=4). Visually-evoked potential (VEP) recordings further showed that the magnitude of cortical responses in Ten-m2 KOs (n=3) was decreased, compared to WTs (n=13), most noticeably for ipsilateral VEP responses ($p<0.05$, multivariate ANOVA). Ten-m2 KO VEP responses also displayed an increased latency and duration ($p<0.05$, multivariate ANOVA). A visual discrimination task was specifically designed to challenge the dorsal visual field of these animals. Here, Ten-m2 KOs (n=9) showed a specific impairment in their ability to discriminate between dorsally-located visual stimuli ($p<0.005$, Mann-Whitney U-test), compared to WTs (n=11). This is consistent with both the ventral retinal deficit and data regarding cortical function in these mice. Together, these data highlight the importance that Ten-m2 has in regulating the formation of functional binocular circuits in the mouse visual system.

POS-MON-002

EPIGENETIC REGULATION OF MEF2C AND HDAC-5 IN MOUSE VISUAL CORTEX BY ENRICHED ENVIRONMENT

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Purpose: Recent studies have shown that the persistence of enriched environment (EE) induces synaptic plasticity in the neuronal circuitry during brain development. In this study we investigated the interplay between EE and epigenetics that organize experience-induced plasticity in the visual cortex (VC). **Methods:** C57BL/6J mice were randomly assigned to standard conditions (SC) or EE conditions 2-3 days before birth. Under SC, mice were singly-housed in a basic cage without any enriched objects until postnatal 56 (P56). Whereas, mice assigned to EE were housed in a Perspex cage containing a variety of objects until P56. Golgi-Cox staining was performed on VC of SC (n=3) and EE mice (n=3) to assess dendritic differences. The mice VC of SC (n=3) and EE (n=3) were dissected for RNA extraction which were examined to validate expression levels of synaptic plasticity related genes: Mef2C, HDAC-5, ApoD, ARC, Egr1, Fos, Per2, TMEM, 176a and TrkA in Real-time quantitative PCR (qPCR) and Chromatin Immunoprecipitation (ChIP-qPCR). **Results:** Golgi-Cox staining revealed enhanced morphological dendritic outgrowth in P28 EE mice, which is not observed in P56 EE mice. Mef2C and TrkA were significantly up-regulated in P28 EE, whilst HDAC-5 was significantly down-regulated after EE in qPCR analysis. ChIP-qPCR showed reduced interaction between HDAC-5 and Mef2C promoter and increased level of acetylated histones in EE. **Conclusion:** These finding indicate that the possibility of the interplay between EE and epigenetics in the VC, leading to new therapies targeting at epigenetic factors for recovery of function in developmental and learning disorders.

POS-MON-003

EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON REVERSAL LEARNING AND HIPPOCAMPAL DENDRITES IN EPHRIN-A2-/- MICE

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Repetitive transcranial magnetic stimulation (rTMS) is thought to facilitate brain plasticity. However, few studies address anatomical changes following rTMS in relation to behaviour. **Purpose** This study examined effects of rTMS on hippocampal dendritic spine densities and reversal learning in a two-choice visual discrimination task comparing wild-type mice and ephrin-A2-/- mice, a model with abnormal neural connectivity and impaired reverse learning strategy (1). **Methods** Using a Y-maze, mice were trained to associate food rewards with one visual stimulus. After learning this association, the rewarded stimulus was switched for the previously incorrect stimulus (reversal learning phase). Complex waveform, subthreshold rTMS was applied daily to 50% of mice in the reverse phase. Mice were terminally anesthetised after 35 days in the reversal learning phase and brains stained with Golgi. Results Behavioural results did not support hypothesised reverse learning deficits observed previously (1) and found no other differences between genotypes or stimulation conditions. Mice of both genotypes were slow to learn the initial learning task and few mice learned the reversal learning task. Analysis of Golgi-stained sections from ephrin-A2-/- mice revealed increased dendrite length and a trend towards spine density increasing in CA1, but decreasing in the dentate granular region following rTMS. **Conclusion** rTMS had no effect on learning behaviour in WT or KO mice despite inducing anatomical changes in the hippocampus. Comparison with previous studies suggests motivation confounded learning results, as mice were food-deprived to lose 10% of their weight, instead of 20% as previously. (1) Arnall, S., Cheam, L.Y., Smart, C., Rengel, A., Fitzgerald, M., Thivierge, J.P., Rodger, J. (2010). Behavioural Brain Research, 209, 109-113.

POS-MON-004

DEVELOPMENTAL VITAMIN D DEFICIENCY IS ASSOCIATED WITH ENHANCED SPONTANEOUS HYPERLOCOMOTION THAT IS AMELIORATED BY AMPHETAMINE PRE-TREATMENT

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Purpose:PET studies indicate that schizophrenic patients are more vulnerable to the effects of amphetamine. Developmental Vitamin D (DVD) deficiency is an environmental risk factor for schizophrenia. In rats, DVD-deficiency leads to an increased susceptibility to the dopamine (DA) releasing effects of amphetamine. This vulnerability is thought to be due to an underlying endogenous dopaminergic sensitization. The aim of this study was to examine whether DVD-deficiency in rats would lower the threshold of amphetamine-mediated DA sensitization. **Methods:**DVD-deficient (n=68) and Control (n=72) Sprague Dawley rats were administered either a Full (n=48), Intermediary (n=46) amphetamine or Saline (n=46) treatment regime, three times per week for three weeks. After a 28d withdrawal period, sensitization was assessed by locomotor response to a low dose amphetamine challenge. Prepulse inhibition (PPI) to an acoustic startle response, novelty-induced hyperlocomotion and DA transporter (DAT) binding in the Nucleus accumbens (Nacc) were also assessed. **Results:**At baseline, DVD-deficiency enhanced novelty-induced hyperlocomotion ($p < 0.01$). Although the current amphetamine pre-treatment regime did not result in sensitization, it did selectively decrease novelty-induced hyperlocomotion in DVD-deficient rats ($p < 0.01$). PPI and DAT density were unaffected by DVD-deficiency or amphetamine pre-treatment. **Conclusion:**The absence of sensitization seen in this study may have been due to source of outbred rat strain, time of amphetamine administration, associative components or testing paradigm. However, DVD-deficiency enhanced spontaneous hyperlocomotion, which replicates previous findings. The fact that this was selectively decreased by prior amphetamine treatment is consistent with the idea that DVD-deficiency induces an underlying DA abnormality.

POS-MON-005

CREATING THE CORTEX: COMPUTATIONAL MODELLING OF THE PATTERNING OF GENES UNDERLYING CORTICAL AREA DEVELOPMENT

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The cerebral cortex is divided into many functionally specialised areas. The development of these areas is dependent on the coordinated expression of several genes. In the embryonic mouse telecephalon, *Fgf8* is expressed at the anterior pole while the transcription factors *Emx2*, *Pax6*, *Coup-tfi* and *Sp8* are expressed in gradients along the anterior-posterior axis. Manipulating the expression of each of these genes during development alters the positions of cortical areas in the adult. Qualitative data also shows that these genes regulate each other's expression during development so that they form a gene regulatory network with many feedback loops. However, it is currently unknown which of these regulatory interactions are critical to generating the correct expression patterns to lead to normal cortical development. We have developed a computational model of this system to identify regulatory networks consistent with experimental data and to predict which interactions are critical to correct anterior-posterior patterning. We previously used a Boolean model, with few free parameters, to do a large-scale screen of many possible networks and identify those with wild type steady states (Giacomantonio and Goodhill, 2010, *PLoS Comput Biol*, 6:e1000936). In the present work, we transformed this subset of Boolean models into more realistic ordinary differential equation models. The continuous expression levels of the differential equation formalism allowed us to simulate additional mutant experiments to further narrow the set of networks consistent with experimental data. While we found that multiple networks perform equally well in reproducing almost all the experimental data, these networks have common elements in their structure.

POS-MON-006

MYCBP2 CONTROLS GUIDANCE OF ROBO2 AXONS DURING DEVELOPMENT OF THE MURINE OLFACTORY SYSTEM

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Purpose: Robo and Slit molecules are crucial axon guidance factors during the development and regeneration of the central nervous system. However, the molecular interactions that these molecules take part in to shape the axon tracts in the CNS remain largely unknown. MYCBP2 is a strongly conserved E3-ubiquitin ligase that regulates axon and synapse development through interactions with multiple signaling pathways. **Methods:** To examine the role of Mycbp2 in olfactory axon guidance we performed immunofluorescence assays on Mycbp2 mutant embryos at 17.5dpc. **Results**: Here I describe how a sub-population of ROBO2 expressing olfactory sensory neurons is severely misguided along the dorsoventral axis of the olfactory bulbs in Mycbp2 loss of function mice, in a pattern strikingly reminiscent of that described for Slit1 and Robo2 mutant mice. We observed a significant loss of innervation in a large dorsal domain in the olfactory bulb. In addition, we showed that these dorsal Robo2 expressing neurons do not die, but instead appear to stall in the ventral outer nerve layer, where they fail to refasciculate with homotypic dorsally targeting axons. In addition we found a strong genetic interaction between Mycbp2 and Robo2 in double mutant mice. **Conclusion:** Altogether, these data suggest that Mycbp2 controls the guidance of ROBO2 expressing neurons during development of the olfactory system and provide important new insights into the genetic cascades that regulate axon guidance processes in the CNS controlled by classic guidance factors ROBO and SLIT.

POS-MON-007

DCC FUNCTIONS IN MULTIPLE ASPECTS OF CORPUS CALLOSUM DEVELOPMENT

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The corpus callosum is a large fibre tract that connects the cerebral hemispheres. Corpus callosum development commences embryonically and continues after birth with multiple mechanisms facilitating axon growth across the telencephalic midline. One molecule implicated in this process is DCC (Deleted in Colorectal cancer), a transmembrane receptor, that binds the guidance ligands Netrin1 and Draxin. DCC can also interact intracellularly with another transmembrane guidance receptor called Robo1 via their P3 and CC1 intracellular domains, respectively. **Purpose:** To investigate the role of the DCC P3 domain throughout callosal development using the mouse model, DCC^{Kanga}, which lacks this domain. **Methods:** Immunohistochemical phenotypic analysis of the DCC^{Kanga} mouse was performed at embryonic, postnatal and adult ages. The phenotype of the DCC^{Kanga} mouse was compared with the *DCC* knockout mouse, which is known to display defects in corpus callosum formation. The development of axonal tracts, cortical lamination and glial development were assessed in both mutants. **Results:** Both homozygous DCC^{Kanga} mice and *DCC* knockout mice display similar embryonic phenotypes, where the corpus callousm is completely absent ($n \geq 3$). DCC^{Kanga} mice also display this phenotype into adulthood ($n \geq 3$). Both mouse lines also display defects in midline glial development, telencephalic fusion and cingulate pioneering axon pathfinding, but cortical lamination occurred normally ($n \geq 3$). **Conclusion:** These results suggest that the DCC P3 domain is required for formation of the entire corpus callosum across embryonic and postnatal development. Furthermore, DCC appears to function in multiple aspects of callosal development, with the P3 domain essential for DCC function. Importantly, these findings provide insight into the mechanisms that underlie brain wiring and may shed light on human congenital disorders of corpus callosum formation.

POS-MON-008

AUSTRALIAN MARSUPIALS: A NOVEL MODEL OF NEUROMUSCULAR JUNCTION DEVELOPMENT?

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Purpose: The somatic neuromuscular junction (NMJ) is a valuable experimental model of synapse formation. Such experiments are most commonly performed on placental mammals (e.g. mouse), where all four limbs develop on a similar timescale. By contrast, some Australian marsupials have very segmented limb development, where forelimbs are precociously developed to assist the journey to the pouch, while hindlimbs are comparatively unformed. We have compared the morphological development of the NMJ in kangaroo hindlimbs and forelimbs, to investigate how NMJ development proceeds in an animal with this novel developmental pattern. **Methods**: Three Western Grey Kangaroo neonates aged postnatal day (P) 0, 26 and 100 were ethically sourced from licensed shooters in South-Western Australia. 5 forelimb and 4 hindlimb muscles from each specimen were embedded, cryosectioned, and stained immunohistochemically for NMJ compartment proteins. **Results**: Confocal micrographs of adult kangaroo NMJs revealed an unusual oval endplate with a internal perforation at its centre. When markers of NMJ development described in rodents (e.g. neurofilament distribution, ACh receptor clustering) were analysed in P0 neonates, forelimb NMJs were considerably more developed than hindlimb junctions. However, between P0 and 100, NMJs in kangaroo forelimbs developed at a slower rate than hindlimb NMJs, so that all limbs were similarly developed by P100. **Conclusions**: Our preliminary data indicates that the segmented limb development in Western Grey Kangaroos is reflected at the level of the NMJ. This segmented NMJ development, combined with the relative accessibility of kangaroo neonates (which undergo a large degree of maturation in the pouch), present novel opportunities for studying synaptic development compared with placental mammals.

POS-MON-009

A HIGH-THROUGHPUT MICROFLUIDICS CHAMBER FOR EXAMINING THE CHEMOTACTIC RESPONSE OF NEURONAL GROWTH CONES

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During nervous system development, growth cones use many types of molecular guidance cues to steer them towards their targets. Some of the most important of these take the form of concentration gradients. However, the methods available for studying growth cone chemotaxis in vitro have so far been quite limited. Three-dimensional collagen gel assays produce only shallow gradients (< 1% change in concentration per 10 microns), while the "pipette" or "growth cone turning" assay produces only steep gradients (~ 10% change). Here we present a novel flow-based chemotaxis chamber based on microfluidic technology in which growth cones can be guided by gradients varying continuously from very shallow to very steep. To provide a proof of concept for this assay we have so far shown significant turning of P2 rat superior cervical ganglion (SCG) neurites over 1 hour up a gradient of Nerve Growth Factor (NGF) of steepness ~ 5% ($n = 13$, $p < 0.05$ compared to control), mimicking results of pipette assays. We have also shown biased growth (without turning) of SCG neurites up (53%) versus down (26%) an NGF gradient of steepness ~ 1% over 12 hours ($n = 141$ total), mimicking results of collagen gel assays. This ability to interpolate between different gradient steepnesses within the same assay will allow quantitative variations in the response strategies of growth cones to shallow versus steep gradients to be examined, without the confounding effect of needing different types of assays to produce the different steepnesses.

POS-MON-010

THE ROLE OF INTERHEMISPHERIC CONNECTIONS IN THE FORMATION OF FUNCTIONAL CORTICAL AREAS

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The corpus callosum connects associated functional areas between the two cerebral hemispheres. People with partial or complete agenesis of the corpus callosum (ACC) exhibit a spectrum of cognitive, motor and sensory deficits. Little is known about whether correct callosal targeting influences the formation of functional cortical areas. Here we investigated this in BTBR mice, which display ACC and are currently used as autism models because they display social and behavioural deficits. Purpose: To investigate whether callosal axon targeting is required for cortical arealization. Methods: The following analyses were performed on BTBR mice using both C57Bl/6 and LP/J mouse strains as controls. Cortical lamination and corpus callosum formation were analysed by immunohistochemistry as well as by dMRI tractography. Cortical arealization was examined by in situ hybridization using region-specific probes and cytochrome oxidase staining to label the somatosensory barrel field. To verify thalamocortical and corticothalamic connectivity, dMRI tractography and DiI tract tracing were performed. Results: BTBR mice displayed normal cortical lamination but complete ACC and partial agenesis of the hippocampal commissure ($n \geq 3$). Arealization, as assessed by molecular markers and thalamocortical and corticothalamic connectivity, were normal in BTBR mice ($n = 3$). The relative position and size of the somatosensory barrel field was normal in BTBR mice ($n \geq 4$ for all strains, $p \leq 0.05$, Student's t-test). Conclusion: The early formation of sensory and motor areas in the mouse cerebral cortex does not require callosal connectivity to occur.

POS-MON-011

REGIONAL VULNERABILITY OF SOMATOSTATIN-POSITIVE INTERNEURONS FOLLOWING INTRAUTERINE GROWTH RESTRICTION

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Purpose: Intrauterine growth restriction (IUGR) can result in abnormal brain development, leading to neurodevelopmental sequelae such as learning difficulties and intellectual and cognitive deficits in childhood. Abnormalities in cortical developmental likely contribute to alterations in these higher brain functions. In this study we investigate the effects of IUGR on subpopulations of interneurons in the cerebral hemispheres in the fetal guinea pig. **Methods:** At 30dg (term ~ 67 days of gestation, dg), chronic placental insufficiency was induced via partial ablation of the uteroplacental blood vessels using diathermy to produce IUGR fetuses ($n=8$); controls ($n=6$) were from sham-operated animals. At 52dg, fetal brains were stained immunohistochemically to identify somatostatin (SST)- and calretinin (CR)-positive interneurons in the cortex, hippocampus and basal ganglia; areal density was determined and expressed as cells per mm^2. **Results:** There was a decrease in the density of SST-positive interneurons in the frontal ($p<0.05$), parietal ($p<0.05$) and occipital ($p<0.01$) cortices and the stratum oriens of the hippocampus ($p<0.05$) in IUGR fetuses versus controls; there was no difference ($p>0.05$) in the caudate-putamen. The density of CR-positive cortical interneurons was not affected by IUGR. **Conclusion:** These results suggest a regional vulnerability of SST-positive interneurons following IUGR. Furthermore, CR-positive cortical interneurons are relatively resistant to this form of prenatal compromise. The long-term neurofunctional implications of these differential effects require further examination.

POS-MON-012

UNILATERAL, BUT NOT BILATERAL, EARLY SENSORY DEPRIVATION DISRUPT THE PATTERN OF CALLOSAL PROJECTIONS

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The corpus callosum carries axons that connect both cortical hemispheres. During the first postnatal week callosal axons from neurons of the barrel cortex cross the midline and arborise in layers 2/3 and 5 to form a dense projection between S1 and S2, known as region specific callosal projection (RSCP). **Purpose:** To determine whether sensory deprivation affects callosal projections from the barrel cortex to the contralateral hemisphere and whether unilateral vs. bilateral whisker deprivation show similar or different effects in callosal projections. **Methods:** Mouse embryos were electroporated *in utero* with eYFP in the right hemisphere at embryonic day 15.5 to label callosal neurons. Pups showing strong and defined fluorescent patches underwent unilateral ($n = 4$) or bilateral ($n = 4$) plucking of mystacial whiskers between P0 and P8. Fluorescent images were obtained from P10 coronal sections at the level of the S1 barrel field, and the axonal innervation and arborisations were quantified in the S1/S2 border region (RSCP) and S1 across cortical layers using ImageJ software. **Results:** Animals with unilateral plucking of whiskers displayed a decrease of around 50% in axonal ramifications in the RSCP ($p < 0.05$, Student's t-test), but showed no difference in S1, as compared with controls. Surprisingly, animals that underwent bilateral plucking of whiskers showed levels of contralateral innervation similar to control animals and differed from unilateral individuals in layers 2/3 and 5 of the RSCP ($p < 0.05$, Student's t-test). **Conclusion:** These results suggest that unilateral deprivation of sensory input affects the targeting of callosal axons and that similar levels of activity in both hemispheres may be required for the normal targeting of callosal projections.

POS-MON-013

MULTIPLE SLITS REGULATE THE DEVELOPMENT OF MIDLINE GLIAL POPULATIONS AND THE CORPUS CALLOSUM

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The Slit molecules are chemorepulsive ligands that regulate axon guidance at the midline of both vertebrates and invertebrates. In mammals, there are three *Slit* genes, but only *Slit2* has been studied in any detail with regard to mammalian brain commissure formation. **Purpose:** To understand the relative contributions that *Slit* proteins make to the formation of the largest brain commissure, the corpus callosum. **Methods:** Diffusion magnetic resonance imaging (dMRI), *in situ* hybridisation and immunohistochemistry were used to analyse Slit and Robo mutant brains. The following strains were analysed Slit1, Slit2, Slit3, Slit1/Slit2, Slit1/Slit3 and Robo1. We also performed *in vitro* collagen gel assays to examine the axonal guidance response of axons from Robo1-/- mice to Slit2. **Results:** Analysis of single mutations in Slit family members revealed corpus callosum defects (but not complete agenesis) in 100% of *Slit2-/-* mice (n=3) and 30% of *Slit3-/-* mice (n=10), whereas 100% of *Slit1-/-; Slit2-/-* mice (n=3) displayed complete agenesis of the corpus callosum. Collagen gel experiments demonstrated that Slit2 was sufficient for the guidance activity mediated by Robo1 in pre-crossing neocortical axons. Investigation of the glia in these strains revealed defects in the development and dorso-ventral positioning of the indusium griseum glia in multiple *Slit* mutants. **Conclusion:** These results revealed a previously unknown role for *Slit1* in corpus callosum development. Furthermore, *Slits* regulate callosal development via both classical chemorepulsive mechanisms, and via a novel role in mediating the correct positioning of midline glial populations.

POS-MON-014

ADULT ZEBRAFISH CNS NEURONS ARE CAPABLE OF EXTENDING NEURITES ONTO CRYOSECTIONS OF MAMMALIAN CNS TISSUE IN TISSUE CULTURE

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Purpose: The adult mammalian central nervous system (CNS) exhibits a very limited capacity for axonal regeneration after injury. However, the situation is very different in lower vertebrates such as teleost fish. These species are capable of regenerating severed CNS axons and re-establishing connections to their targets. A greater understanding of the mechanisms involved in this repair and regeneration in sub-mammalian species may give an insight into how we may also repair the adult mammalian CNS. **Methods**: 10µm-thick cryostat sections of frozen cerebral and cerebellar hemispheres were picked up on sterile poly-L-lysine coated plastic coverslips and placed in 4-well tissue culture plates. Preparations of adult zebrafish CNS neurons were plated onto these sections and incubated at 28.5°C in an atmosphere containing 1.5% carbon dioxide. After 14 days of incubation, the cultures were fixed in 2% paraformaldehyde and double immunolabelled with anti GAP-43 (for growing neurites) and anti-GFAP (for glial cells in the sections). **Results**: It was found that adult zebrafish CNS neurons were capable of extensive neurite regeneration on the poly-L-lysine coated plastic coverslips (n=6). It was further observed that many of these neurites were capable of growing over the cryostat sections of the rat cerebral and cerebellar tissues. **Conclusion**: This growth has not been observed when adult mammalian CNS neurons have been used in similar experiments. This indicates that adult zebrafish CNS neurons do not respond to the inhibitory factors normally found in mammalian CNS tissue. Whether this is due to the lack of appropriate receptors to the inhibitory molecules generally found in mammalian CNS requires further research.

POS-MON-015

INNERVATION OF SENSORY HAIR CELLS BY HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS IN VITRO

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Purpose: The loss of sensory hair cells and spiral ganglion neurons (SGNs) that occurs with profound hearing loss is irreversible, and SGNs are also observed to progressively degenerate as the period of deafness increases. Stem cell therapy for replacement of lost SGNs thereby offers a potential approach to improve the hearing of profoundly deaf individuals. It has been shown that human embryonic stem cell (ESC)-derived neurons can be generated *in vitro* by treatment with Noggin and Y27632, and these neurons can innervate early post-natal cochlear explants. This project aims to investigate the formation of peripheral synaptic connections between ESC-derived neurons and sensory hair cells. **Methods:** ESC-derived neurons were co-cultured with hair cell explants from either P2/3 (n=8) or P5/6 (n=10) rats, for 1-2 weeks *in vitro*. Innervation of hair cells including synapse formation was examined using immunofluorescence labelling and confocal microscopy. **Results:** Human ESC-derived neurons extended neurites toward and along the rows of sensory hair cells in all co-cultures. Extensive numbers of synapsin 1-positive pre-synaptic terminals were found between hair cells and ESCs and also between ESCs in co-cultures only after 2 weeks *in vitro*. **Conclusion:** These results show that neurites from human ESC-derived neurons can reach hair cells within 7 days in co-culture, however the ability of human ESC-derived neurons to synapse on sensory hair cells *in vitro* may depend on the age of the tissue used and time in culture. This assay will be used to characterise the timing of synapse formation between human ESC derived neurons and their peripheral (hair cell) and central (cochlear nucleus) targets *in vitro*.

POS-MON-016

PHENOTYPIC EFFECTS OF NEUROTROPHIC FACTORS ON FOREBRAIN SELF-REPAIR POST-EXCITOTOXIC INJURY

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INTRODUCTION The Subventricular Zone (SVZ) lining the Lateral Ventricles of the striatum is a neurogenic region which generates migratory neuroblasts throughout adult life. Upon insult, neuroblasts born from this proliferative zone respond to chemo-attractants in the damaged striatum and migrate to the site of injury. Due to a lack of neurotrophic queues in the damaged striatum, the majority of migrating cells die over the long term. Neurotrophic factors present in the adult and developing brain are defined by their ability to influence cell proliferation, chemotaxis, neuroprotection and restoration in the brain. Glia cell-derived neurotrophic factor (GDNF) is a well studied protein and its positive therapeutic potential in other neural diseases and injuries has been previously shown. Whether these neuroprotective effects can be translated to the more clinically relevant post-administration of GDNF, and how GDNF influences phenotypic acquisition of injury-responsive neuroblasts has yet to be demonstrated. **METHOD** In order to obtain a highly detailed analysis of the forebrain injury self-repair mechanism and analyse the therapeutic potential of GDNF, rats were lesioned via intrastriatal surgery with quinolinic acid. Post-lesioning, trophic support was administered intrastriatally via a lentiviral construct over-expressing GDNF. **RESULTS** BrdU+ cells in the striatum at 4 weeks were significantly increased ($P<0.01$) when comparing lesioned to unlesioned animals. BrdU+/NeuN+/DARPP32+ labeling revealed no triple labeled cells. However in the GDNF supported animals, at 4 weeks post-lesion BrdU+/NeuN+ cells were increase ~5 fold ($P<0.01$) in the lesioned area when compared to lesion alone. **CONCLUSION** In conclusion, newly born cells migrate into the damaged striatum where none acquire a functional striatal neuronal phenotype (DARPP32) after 6 weeks. GDNF treated animals have increased BrdU+/NeuN+ cells suggesting GDNF might positively influence neuronal differentiation or positively influence the survival of neuronal differentiated cells.

POS-MON-017

AXON GUIDANCE IN A THREE DIMENSIONAL COLLAGEN MATRIX WITH CHEMICAL GRADIENTS GENERATED USING A SIMPLE DUAL COMPARTMENT DIFFUSION CHAMBER

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The generation of chemical guidance cue gradients is essential for axon guidance and cell migration research. These applications require maintenance of a stable gradient which should also permit either live imaging via fluorescent proteins or immunostaining. We present a simple two-compartment chamber for the generation of chemical gradients in a three dimensional collagen matrix. Gradient steepnesses of 0-5% are achieved within 6 hours post setup. The technique is spatially well-defined, enabling high reproducibility of both gradient steepness and guidance cue concentration. Demonstration experiments using gradients of Nerve Growth Factor (NGF) elicited chemoattraction of dorsal root ganglion neurites over 48 hours compared to NGF plateaus (n=42, n=7 respectively, p=0.042), while gradients of Slit2 elicited chemorepulsion of olfactory bulb neurites over 72 hours compared to Slit2 plateaus (n=48, n=11, p=3.7 X 10-5). This method provides a rapid and reproducible system for the quantitative analysis of chemotropism with minimal setup cost.

POS-MON-018

AN INTRACELLULAR DOMAIN FRAGMENT OF THE P75 NEUROTROPHIN RECEPTOR INCREASES TRK RECEPTOR LIGAND BINDING AND SIGNALING BY AN INSIDE-OUT ALLOSTERIC MECHANISM

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Purpose: The common neurotrophin receptor ($p75^{NTR}$) enhances the response of tropomyosin-relatated kinase (Trk) receptors to neurotrophins, resulting in high affinity binding. The precise mechanism by which Trk and $p75^{NTR}$ co-operate to enable high affinity binding remains unresolved. **Methods:** $p75^{NTR}$-Trk interactions were investigated in HEK293, PC12 cells using receptor constructs or synthetic receptor-region mimic peptides and cultured embryonic E13 motor neurons (MNs) using, colorimetric viability assays, neuronal differentiation, immunobloting, immunoperipitation, flow cytometry and receptor ligand binding techniques. **Results:** Here, we report that a 29 amino acid juxtamembrane region of p75 intracellular domain ($p75^{ICD}$) interacts with, and is essential for, the enhanced signaling in survival and differentiation via TrkA and TrkB receptors, and blocking $p75^{ICD}$ generation inhibits optimal TrkA function (n=7). In addition we found that a peptide of $p75^{ICD}$ (c29) spanning the TrkA binding site, promotes TrkA-mediated differentiation and survival following growth factor withdrawal in PC12 cells (n=6). Moreover, c29 promotes survival of cultured MNs, but only in the presence of TrkB co-activation (n=9). We show that both $p75^{NTR}$ construct and c29 enhance the amount of NGF that binds to TrkA expressing cells, by increasing their binding capacity. The deletion of this 29 amino acid juxtamembrane region of $p75^{NTR}$ is sufficient to abolish this high-affinity binding. **Conclusion:** We conclude that $p75^{ICD}$ potentiates high-affinity binding and signaling via TrkA through its 29 amino acid juxtamebrane region, possibly through an allosteric mechanism resulting in structural conformation changes within Trk receptors.

POS-MON-019

NDFIP1 SUPPORTS DENDRITIC ARBOR AND SPINE DEVELOPMENT

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Ndfip1 (Nedd4 family interacting protein 1), an adaptor molecule for the E3 ubiquitin ligase Nedd4, is expressed throughout the mouse brain during development and in the adult. Peak Ndfip1 expression during the first post-natal weeks coincides with maturation of the dendritic arbor and synaptogenesis, suggesting a possible role for Ndfip1 in these processes. Purpose: To use mice deficient in Ndfip1 to investigate the function of this protein during corticogenesis. Results: While conditional deletion (CKO) of Ndfip1 in neural precursors during corticogenesis had no effect on cortical layering or total neuronal numbers (n=3; postnatal day 30: control 2072 ±129; CKO 2103 ±137; postnatal day 60: control 2101 ±9; CKO 2071 ±77), pyramidal neurons conditionally-deleted for Ndfip1 exhibited aberrant morphology. Atrophic characteristics of these neurons (n=16) included stunted dendritic arbors (sum basal dendrite lengths: CKO -1010 ±111 µm; Control - 1483 ±160 µm, p=0.02), reduced dendritic spine numbers (CKO -107.5 ±12.5; Control – 158.3 ±16, p=0.02) and shrunken compacted somata. In culture, Ndfip1 knockout neurons exhibited excessive sprouting of dendrites up to 120 µm from the cell body and ectopic expression of Ndfip1-GFP in wildtype neurons revealed accumulation of fluorescent protein at dendritic branch points and at the base of spines. Western blot analyses indicated that activity of the PI3K-Akt pathway is misregulated in the cortex of Ndfip1 knockout mice. [All results expressed as mean ±SEM]. Conclusion: Lack of Ndfip1 leads to altered dendritic morphology and reduced spine density on pyramidal neurons, suggesting that Ndfip1 is essential for regulating synaptic connectivity.

POS-MON-020

SELECTIVE SEROTONERGIC EXCITATION OF CALLOSAL-PROJECTION NEURONS

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Background: Serotonin (5-HT), acting at inhibitory $5\text{-}HT_{1A}$ (1A) and excitatory $5\text{-}HT_{2A}$ (2A) receptors, modulates layer 5 pyramidal neuron (L5PN) excitability in the medial prefrontal cortex (mPFC). 2A receptors are implicated in schizophrenia and are targeted by atypical antipsychotic drugs. However, only a minority of L5PNs appear to be functionally excited by 5-HT. **Purpose:** We tested the hypothesis that 5-HT responsiveness in L5PNs is correlated with their axonal projections. **Methods:** Retrograde labeling with fluorescent beads was used to identify L5PNs with commissural projections to the contralateral cortex (COM neurons) and L5PNs projecting to the pons (CPn neurons). Subsequent whole-cell recordings of labeled neurons in acute slice preparations allowed us to test serotonergic responsiveness in these two projection-specific L5PN populations. **Results:** 5-HT responsive COM neurons (n = 24 out of 26) showed 5-HT-induced increases in excitability, while all CPn neurons (n = 17) were inhibited by 5-HT. COM neurons were physiologically and morphologically distinct from CPn neurons in having higher input resistances and less-branched apical dendrites. Additional preliminary experiments found that labeled COM neurons in layer 2/3 of the mPFC (n = 9) were also excited by 5-HT, while non-labeled L2/3 pyramidal neurons (n = 4) were 5-HT-inhibited. Serotonergic excitation of COM neurons was blocked by the 2A receptor antagonist MDL 11939, confirming a role for 2A receptors in cortical excitation. **Conclusion:** Our data demonstrate that 5-HT selectively excites COM projection neurons in the mPFC via $5\text{-}HT_{2A}R$ activation, suggesting that activity in these neurons may contribute to the psychotropic effects of hallucinogens and to psychosis.

POS-MON-021

DESENSITIZATION OF ACUTE μ-OPIOID RECEPTOR SIGNALING MEASURED USING A CONTINUOUS MEMBRANE POTENTIAL ASSAY

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Purpose: Opioids have unique analgesic and rewarding properties, largely mediated by activation of the μ-opioid receptor (MOR). Acute agonist exposure produces MOR desensitization, usually measured with biochemical assays or relatively invasive electrophysiological techniques. We sought to determine whether measurement of membrane potential in intact cells is a suitable assay for examining changes in MOR signaling in a cell line endogenously expressing G protein-gated K channels. **Methods:** AtT20 cells stably transfected with mouse MOR were grown in 96 well plates and serum starved overnight. Cells were incubated with a proprietry membrane potential-sensitive dye (Molecular Devices, Blue dye) and continuous fluorescence readings obtained using a Flexstation 3. The degree of desensitization was quantified using a high concentration of agonist added at various time points after the desensitizing stimulus. Heterologous desensitization was assessed with SRIF. **Results:** Morphine and met-enkephalin (ME) produced rapid decreases in fluorescence with EC50s of 28±5nM and 3±4nM respectively (n=5-6). Desensitization was observed with both agonists. At equilibrium, morphine (1μM) produced a 71±1% inhibition of the hyperpolarization produced by a subsequent application of 10μM morphine (t/2 340s). The response to SRIF (1μM) was inhibited by 29±1%, with an identical timecourse. Met-enkephalin (1μM) produced a 58±2% inhibition of a subsequent application of ME (10μM), and a 47±3% decrease in the response to SRIF (n=5). Pretreatment of cells with the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (1μM) did not significantly affect morphine potency, efficacy or desensitization. **Conclusion:** Our data reinforce the idea that MOR desensitization is rapid, and not necessarily dependent on PKC. Non-invasive measurement of membrane potential may prove a powerful technique to examine acute changes in MOR signaling.

POS-MON-022

EXCITABILITY OF HUMAN VENTRAL HORN NEURONS DURING EARLY FOETAL DEVELOPMENT

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Much of what we know about the development of ventral horn neurons (VHNs) comes from studies in rodents. This work has shown the intrinsic properties of VHNs change dramatically during late foetal and early postnatal development. No data are available for VHNs at any stage of human foetal development. Purpose: To examine the intrinsic properties of human VHNs during early foetal development. Methods: Transverse slices (400 μm thick) were obtained from the spinal cords of terminated human foetuses (10-18 weeks gestation; WG). Whole cell recordings were made from visualized VHNs at 32°C using a KCH_3SO_4-based internal. Results: We recorded from 55 VHNs at three gestational age groups (10-12, 13-15, ≥ 16WG). Resting membrane potential became more hyperpolarized (-54.7 ± 2.6 vs. -64.6 ± 2.7 mV) and input resistance decreased (875 ± 120 vs. 331 ± 88 MOhm) over the time points examined. Action potentials (APs) were elicited, during current injection, in 14/21 VHNs at 10WG and 22/23 at ≥16WG. AP afterhyperpolarization changed significantly during development (-8.3 ± 2.2 vs -18.8 ± 2.0 mV). Sustained current injection resulted in tonic AP discharge in most VHNs recorded at each age (12/19 at 10WG and 15/23 at ≥16WG). Conclusion: These data suggest human VHNs, some of which will be motoneurons, are electrically excitable by at least 10 WG. This is consistent with the appearance of sustained muscle activity in the very young foetus.

POS-MON-023

DETERMINANTS OF FATTY ACID ACTIVATION OF THE PRONOCICEPTIVE ION CHANNEL TRPA1

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Purpose: The transient receptor potential ankyrin 1 (TRPA1) channel expressed on primary afferent nociceptors detects potentially damaging environmental stimuli such as noxious cold, changes in pH, noxious chemicals and endogenous products of inflammation. TRPA1 is a major target for the development of drugs to treat pain. Many irritant chemicals activate TRPA1 via covalent modification of intracellular N-terminal cysteines. In this study we have investigated activation of TRPA1 by chemically unreactive fatty acids and their derivatives; including N-acyl neurotransmitter/amino acid conjugates. **Methods**: HEK293 cells expressing human TRPA1 under the control of a tetracycline-sensitive repressor were grown in 96 well microplates. Intracellular calcium was measured using the calcium 5 kit from Molecular Devices using a FLEX Station Microplate Reader. Results are expressed as mean±sem of at least 3-5 determinations. **Results**: The covalent TRPA1 agonist cynnamaldehyde activated TRPA1 with an EC_{50} of 20±4μM, producing a maximum change of fluorescence of 407±10%. Arachidonic acid (AA, 30μM) increased fluorescence by 339±105% with a notional EC_{50} of 10±2μM. Anandamide (179±42%), NA-tyrosine (124±20%), NA-dopamine (30±2%), NA-glycine (70±35%) and NA-taurine (93±4%) all increased fluorescence at 30μM. Interestingly, increasing or decreasing the degree of saturation of the fatty acid substantially reduced efficacy to activate TRPA1 compared with AA. Sub-threshold doses of AA (100 nM, 300nM and 1 μM) all increased the maximum response respectively (by 116±2.9%, 126.7±5.9%, 128.5±8.5 %) of TRPA1 to cynnamaldehyde without affecting its potency. **Conclusion**: Our data show that AA is a more potent activator of TRPA1 than other fatty acids or derivatives. AA may also activate TRPA1 via a mechanism distinct from that of cynnamaldehyde.

POS-MON-024

A NOVEL FLUORESCENCE BASED ASSAY OF μ-OPIOID RECEPTOR MEDIATED GIRK CHANNEL ACTIVATION IN ATT-20 CELLS

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Opioid drugs such as morphine are widely prescribed analgesics, however their use is limited due to the development of tolerance and addiction, as well as high variability in individual response. **Purpose**: The development of improved opioid analgesics requires high-throughput functional assays to assess large numbers of potential opioid ligands. In this study, a novel fluorescence-based assay of G-protein coupled inwardly rectifying potassium channels (GIRK) activation was developed, based on a proprietary “no wash” membrane potential assay. **Methods**: AtT-20 cells stably expressing mouse MOR were grown in 96-well microplates and assayed using the Molecular Devices FLIPR membrane potential dye. Dye emission intensity increases on membrane depolarization, and decreases upon hyperpolarization. **Results**: Fluorescence decreased in a dose-dependent manner upon application of range of opioid ligands to the cells (n=5 replicates per ligand). Lowering extracellular K from 5.5 to 2.5 mM increased the maximum opioid responses by 25%. High efficacy agonists produced a decrease of 35% to 40% in total fluorescence, with pEC50s consistent with prior work (fentanyl 8.9, DAMGO 8.3, β-endorphin 7.2, oxycodone 6.7) The maximum effect of opioids was similar to that of somatostatin, which couples to endogenous receptors in AtT-20 cells, or nigericin (100nM), an antibiotic which forms potassium selective pores. Morphine (Emax 30%, pEC50 7.6) and buprenorphine (Emax 19%, pEC50 7.7) were partial agonists. The effects of opioids were prevented by prior treatment with pertussis toxin (200 ng/ml, overnight) and inhibited by naloxone. **Conclusions**: The measurement of GIRK activation by MOR is an effective high-throughput assay for assessing ligand potency and efficacy and may be useful as an additional method for screening novel opioid ligands.

POS-MON-025

AGONIST REGULATION OF 5-HT1B RECEPTOR SIGNALING

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Purpose: Migraine is a recurring and debilitating disorder affecting about 15% of people. Triptans, 5-$HT_{1B/1D}$ receptor agonists, are effective anti-migraine drugs but regular or extended use can lead to a loss of effectiveness. Little is known about how agonists regulate 5-HT_{1B} receptors and thus we created a model system to study the effects of 5-HT and anti-migraine drugs on 5-HT_{1B} receptors in a neuronal environment. **Methods:** Mouse AtT-20 cells were stably transfected with HA-tagged human 5-HT_{1B} receptor cDNA. Recombinant 5-HT_{1B} receptor signaling was assessed using whole cell patch clamp recordings of endogenous G protein-gated inwardly rectifying potassium channels (GIRK). Each data point represents 6 or more cells. Immunohistochemistry was used to detect changes in cell surface receptors upon application of an agonist. The experiment was repeated three times and 6 confocal images were analysed for each condition. **Results:** In an AtT-20 clone expressing 5-HT_{1B} receptors, 5-HT coupled strongly to activation of GIRK, with an EC_{50} of 12nM. Sumatriptan activated GIRK in these cells with an EC_{50} of 60nM. Both 5-HT and sumatriptan produced profound desensitization of 5-HT_{1B} receptor signaling within a few minutes ($\tau \sim 3$min), with an EC_{50} of 24nM and 280nM, respectively. Complete recovery from desensitization was observed after 10 minutes for both 5-HT and sumatriptan. Significant changes of receptor cell surface expression were detected as early as 10 minutes after treatment with 38% reduction by 10μM 5-HT and 18% reduction by 10μM sumatriptan. **Conclusion:** We have shown that desensitization and internalization of the 5-HT_{1B} receptor is rapid and profound. This is the first system, where 5-HT_{1B} receptor-specific processes can be observed in real-time and which thus allows the comparison of multiple events involved in 5-HT_{1B} receptor regulation.

POS-MON-026

IFENPRODIL BLOCKS GLUTAMATERGIC SYNAPSES IN THE BASOLATERAL AMYGDALA BY A PRESYNAPTIC MECHANISM

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Ifenprodil is widely used as a selective blocker of NMDA receptors that are composed of GluN1 / GluN2B heterodimers and has been extensively used to test the role of GluN2B-containing NMDA receptors in learning and memory formation. However, ifenprodil has also been reported to have actions at a number of other receptors, including high voltage-activated calcium channels, adrenoceptors and 5-HT_3 receptors. **Purpose and methods:** In this study we have investigated the actions of ifenprodil at excitatory synapses in the basolateral amygdala (BLA) using electrophysiological recordings from neurons in acute brain slices. **Results:** We found that ifenprodil dose -dependently blocks AMPA receptor-mediated transmission and increases the paired pulse ratio at excitatory Cortical-BLA synapses. This inhibitory action of ifenprodil has an IC_{50} of 10μM and was not occluded by the prior addition of the broad-spectrum NMDA receptor antagonist APV. Whole cell AMPAR receptor mediated currents elicited by application of glutamate to the BLA neurons were unaffected by ifenprodil and spontaneous excitatory current frequency was also reduced by ifenprodil, whereas amplitude was unaffected. Both of these results suggested that ifenprodil inhibited cortical – BLA transmission via a pre-synaptic mechanism. The Adrenoceptor antagonists yohimbine and prazosin, and the $5HT_3$ receptor antagonist Y25130 did not mimic this effect and ifenprodil remained effective following the combined application of these drugs. In contrast, Ifenprodil's effect at these synapses was fully occluded by the P/Q type calcium channel blocker omega – agatoxin however. **Conclusion** We conclude that ifenprodil reduces synaptic transmission in the basolateral amygdala by reducing the probability of release. This effect results from ifenprodil partially blocking P-type voltage dependant calcium channels, rather than by a NMDA receptor-mediated mechanism.

POS-MON-027

ELUCIDATING THE ROLE OF SEROTONIN IN SLEEP-WAKE BEHAVIOUR USING OPTOGENETICS

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Purpose: Serotonin can profoundly effect sleep-wake cycles by altering neuronal oscillations during deep sleep, particularly hippocampal theta oscillations. Optogenetics, a recently developed technique, can greatly improve our understanding of the role of serotonin by providing specific activation of serotonin raphe neurons in freely behaving animals via laser photostimulation of light sensitive ion channels (channelrhodopsin-2). **Methods:** Using the CRE-Lox system with CRE recombinase under control of the serotonin transporter (SERT) promoter, transgenic SERT/CRE mice were injected with adeno-associated virus containing the channelrhodopsin-2-eYFP (ChR2-YFP) construct in the dorsal raphe using stereotaxic injection methods (n=14). **Results:** Using immunocytochemistry and in situ hybridization for CRE, we first showed that ChR2 expression was specific for raphe neurons. The targeted raphe neurons expressed both SERT and tryptophan. Raphe projections to ventral and dorsal hippocampus were immunopositive for YFP seven months post-injection of virus into raphe nucleus, confirming stable viral infection and expression of light sensitive ChR2 (n=7). Photostimulation of ChR2 in brainstem slices elicited action potential firing in current-clamp recordings of raphe neurons (n=3). In vivo polysomnographic recordings are underway following cannula implantation for fibre optic access and photostimulation of raphe nuclei, and electrode attachment (four electrodes in skull for electroencephalographic recording; two electrodes in neck musculature for electromyographic recording; n=3). **Conclusion:** We have successfully transfected the opsin ChR2 specifically in 5-HT neurons of the raphe. These results provide the basis to elucidate the role of serotonin in sleep-wake cycles in freely behaving animals.

POS-MON-028

MECHANISMS OF ACTION OF THE INSECTICIDES, LINDANE AND FIPRONIL, ON GLYCINE RECEPTOR CHLORIDE CHANNELS

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Purpose: Molecular docking studies predict that the insecticides, lindane and fipronil, block GABA type-A receptors ($GABA_A$Rs) by binding to 6' pore-lining residues. However, has never been tested at any Cys-loop receptor. The neurotoxic effects of these insecticides are also thought to be mediated by $GABA_A$Rs, although a recent morphological study suggested glycine receptors (GlyRs) mediate fipronil toxicity in zebrafish. Here we investigated whether human α1, α1β, α2 and α3 GlyR subtypes are sufficiently sensitive to block by either compound as to represent possible neurotoxicity targets. We also investigated the mechanisms by which lindane and fipronil bind to α1 GlyRs. **Methods:** GlyRs were recombinantly expressed in HEK293 cells and insecticide effects were studied using patch-clamp electrophysiology. **Results:** Both compounds completely inhibited all tested GlyR subtypes with IC_{50} values ranging from 0.2 - 2 μM, similar to their potencies at vertebrate $GABA_A$Rs. In agreement with molecular docking predictions, we found that both lindane and fipronil interact with 6' Thrs via hydrophobic interactions and hydrogen bonds. In contrast with predictions, we found no evidence for lindane interacting at the 2' level. We also present evidence for fipronil binding in a non-blocking mode in the anaesthetic binding pocket, and for lindane being an excellent pharmacological tool for establishing the presence of β subunits in αβ heteromeric GlyRs. **Conclusions:** This study implicates GlyRs as novel vertebrate toxicity targets for fipronil and lindane. It also shows that lindane interacts with 6' Thrs whereas fipronil may have both pore and non-pore binding sites.

POS-MON-029

MODULATION OF CANNABINOID RECEPTOR INTERACTING PROTEIN (CRIP1A) EXPRESSION USING SIRNA-MEDIATED KNOCKDOWN AND EFFECTS ON NEURONAL CELLS IN CULTURE

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The primary cannabinoid receptors in the central nervous system are CB_1 receptors. Cannabinoid receptor interacting protein ($CRIP_{1a}$), binds to and interacts with the C-terminal tail of the CB_1 receptor (aa 418-472) and has been shown to suppress the tonic inhibition of voltage-gated Ca^{2+} channels induced by CB_1 receptors in neurons (Niehaus et al., 2007). Activation of CB_1 receptors activates $G_{i/o}$ proteins and therefore inhibits adenylate cyclase, cAMP formation and Ca^{2+} mobilisation and causes phosphorylation proteins in the MAP kinase pathway including ERK1/2. **Purpose:** The effects of cannabinoid receptor activation on the CNS are predominantly mediated by the activation of CB_1 receptors. Therefore $CRIP_{1a}$ provides a new avenue for modulation of the endocannabinoid system in the CNS. **Methods:** Initial studies screened three individual $CRIP_{1a}$-siRNA (n=3) in NG108-15 cells inherently expressing $CRIP_{1a}$. Cells were transfected with $CRIP_{1a}$-siRNA (5nM, 20nM and 40nM) using Lipofectamine RNAiMAX and 48 hours post-transfection, cells were collected for lysate preparation and RNA extraction. **Results:** Western blot analysis showed all three $CRIP_{1a}$-siRNAs to induce significant reduction in $CRIP_{1a}$ protein levels ($p < 0.001$). Furthermore, quantitative RT-PCR analysis of mRNA extracted from NG108-15 cells showed a corresponding reduction in mRNA levels ($p < 0.05$) in two of the three siRNAs screened. Candidate downstream signalling pathways are currently being screened to investigate the effect of decreased $CRIP_{1a}$ on modulation of MAP kinase/ERK activity by maximally effective agonist concentrations. **Conclusion:** These studies provide an in vitro insight into the mechanisms involved in $CRIP_{1a}$ modulation of CB_1 receptors.

POS-MON-030

ACTIVATING BIOAMINE CIRCUITS IN *DROSOPHILA* CONFERS RESISTANCE TO THE VOLATILE ANAESTHETIC ISOFLURANE

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Purpose: General anaesthesia is a routine procedure that causes loss of behavioural responsiveness in all animals and unconsciousness in humans. Although this phenomenon has been known for over 150 years, its mechanisms remain unclear. Whereas GABA has been associated with general anaesthesia, the role of other neurotransmitter systems remains in question. Here, we use the genetically tractable model *Drosophila melanogaster* to investigate the role of bioaminergic systems in modulating anaesthesia phenotypes. **Methods:** We developed an automated paradigm to measure general anaesthesia in flies. Filmed *Drosophila* genetic variants were exposed to increasing isolurane concentrations while being tested for responsiveness to mechanical stimuli, thereby yielding isoflurane EC50 measures for each strain. We used key mutant strains as well as the UAS/Gal4 expression system to control the function of different neurotransmitters in the fly brain. Activation of circuits was achieved by targeting ion channels or the vesicle fusion machinery. **Results:** Our genetic manipulations show no role for GABA in modulating isoflurane anaesthesia in *Drosophila*. Instead, we found that activating dopamine (DA) or octopamine (OA) neurons confers resistance to isoflurane ($p<0.0019$ n=7 $p<0.0001$ n=6 respectively). Genetic manipulations in DA and OA circuits suggest that calcium rather than sodium influx in these neurons produces isoflurane resistance. **Conclusion:** Our data suggest that bioamine circuits, which modulate arousal and sleep in *Drosophila*, also control isoflurane sensitivity in the fly brain. Isoflurane resistance through DA and OA manipulations appears to be mediated by calcium effects at the synapse.

POS-MON-031

CHOLESTEROL LOWERING THROUGH HMG COA REDUCTASE INHIBITORS (STATINS) IMPAIRS LONG TERM POTENTIATION AND INDUCES ANXIETY IN GUINEA PIGS

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Purpose Statins reduce the risk of death from cardiovascular disease, and are prescribed to millions of people worldwide. Recent data shows that people taking statins have an increased risk of psychiatric adverse events such as amnesia, anxiety and aggression. However, there are few experiments investigating the effect of statins on neural function. **Methods** This study aimed to investigate the effect of simvastatin (1mg/kg) and atorvastatin (0.5mg/kg) treatment on memory in an animal model of spatial memory and learning (Morris Water maze), and to investigate their effect on synaptic plasticity using extracellular field recordings of synaptic transmission in area CA1 of freshly prepared hippocampal slices (400µm) in comparison with the effects of acute cholesterol lowering with lipid lowering drugs. **Results** Statin treatment increased the time animals took to find the platform over 5 days, though this result was not significant when compared with control animals. However, thigmotaxis and swimming speed were significantly ($p<0.05$ & $p<0.01$ respectively) elevated in the drug treated groups compared with control animals. Furthermore, Long Term Potentiation (LTP), a key paradigm used to investigate memory changes within the hippocampus was significantly diminished in the presence of 5µM atorvastatin (62%), 5µM simvastatin (48%) and 0.5mg/ml methyl-β-cyclodextrin (MBCD) (30%) ($P<0.05$) when compared with vehicle treated slices. **Conclusion**: Deficits in water maze performance and hippocampal LTP demonstrated here are suggestive of statin induced changes in synaptic plasticity in the hippocampus. Further investigations into acute and chronic statin dosing and their effects on LTP, LTD and receptor populations will help elucidate the mechanism(s) of statin associated amnesia and anxiety.

POS-MON-032

ORGANISATION OF PRIMARY AND SECONDARY SOMATOSENSORY CORTICAL RESPONSES IN THE CAT TO VIBROTACTILE STIMULATION ON THE GLABROUS SKIN OF THE FOREPAW

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Purpose: The cortical organization in the cat's primary (SI) and secondary (SII) somatosensory regions of cortex to vibrotactile stimuli of high and low frequency was investigated using multichannel penetrating electrodes. **Methods:** Sinusoidal vibrotactile stimuli of 20Hz (or 23Hz), 200Hz or combined 20/200Hz (or 23/200Hz) were presented at various amplitude combinations to the glabrous skin of the forepaw of anaesthetized cats (n=3). Multi-unit spike activity was recorded from 64-channel NeuroNexus arrays inserted into contralateral SI and SII, in the region receiving input from the glabrous skin of the forepaw. All procedures were approved by the ACEC of UNSW. **Results:** Both SI and SII exhibit localized regions of excitatory spiking response with respect to the stimulated digit. The response increased correspondingly to the stimulus amplitude. Further, electrode sites neighbouring the excitatory regions were observed to exhibit a small inhibition of the response with the same vibrotactile stimuli. From a given recording site, the multi-unit activity may either be excited or inhibited from baseline depending on the stimulated digit. The inhibition may be restricted at some sites to the pure 20Hz (or 23Hz) or 200Hz sinusoids, but frequently the inhibition was induced by either frequency and by the combined stimuli. **Conclusion:** Vibrotactile stimulation causes both excitation and inhibition in SI and SII, dependent on the spatial relation of the stimulus and recording sites. The inhibition was at a much smaller magnitude compared to the excitatory effect, and may be independent of the stimulus frequency.

POS-MON-033

ALTERED C-TACTILE PROCESSING IN HEAT/ CAPSAICIN-INDUCED ALLODYNIA

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Tactile allodynia is generally considered to be mediated by A-beta afferents. However, stimulation of C-tactile afferents may increase ongoing muscle pain, suggesting a role for these fibres in tactile allodynia (Nagi et al, J Physiol, 2011) We used the heat/capsaicin sensitization model to study C-tactile afferents role in tactile allodynia in healthy subjects as well as in an A-beta denervated subject (G.L.). We used a 3x3 cm thermode to deliver a 45 deg. C heat stimulus to the skin for 5 minutes after which capsaicin cream (0.075%) was applied for 30 minutes. Optimal stimulation of C-tactile afferents (stroking the skin with a velocity of 3 cm/sec) was delivered in the intended allodynia zone (iAZ) and in an untreated control zone. In G.L., fMRI was used to compare cortical sensory processing during brushing in the iAZ and the control zone (3 T, blocks of 3 s brushing and 15 s rest, repeated 100 times). In a two alternative forced choice (2-AFC) psychophysical paradigm in healthy subjects (n = 4) cotton swab brushing in the iAZ was perceived as more unpleasant than in the control zone (ratio of 127:1, $P < 0.001$, binomial distribution). G.L did not perceive brushing in the iAZ as unpleasant. However, she perceived brushing in the iAZ as being significantly weaker than in the control zone (ratio of 10:0, $P < 0.001$, binomial distribution). fMRI in G.L. showed significant activation in several areas including mid anterior orbitofrontal cortex (mid ant OFC) for the contrast control zone minus iAZ. Further, G.L. had activation in inferior frontal gyrus and mid temporal cortex for the contrast iAZ minus control zone. We conclude that C-tactile processing is altered during heat/capsaicin induced allodynia and that A-beta afferents are necessary for perception of allodynia.

POS-MON-034

WHOLE CELL PATCH CLAMP RECORDINGS FROM MUSCLE SPINDLE AFFERENT NEURONS IN INTACT DORSAL ROOT GANGLIA ISOLATED FROM MOUSE

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Muscle spindle afferents play a crucial role in regulating skeletal muscle function and disruption of this afferent signalling is implicated in the development of spasticity. In rodents muscle spindle afferent neurons can be identified by their selective expression of the calcium binding protein parvalbumin (Ichikawa et al., Developmental Brain Research 150: 41–45). **Purpose:** To develop a preparation for making patch-clamp recordings from identified muscle spindle afferent neurons in isolated intact dorsal root ganglia. **Methods:** Experiments used transgenic mice that expressed enhanced green fluorescent protein under the control of the parvalbumin promoter (PVeGFP, Meyer et al., J Neurosci 22:7055–64). Mice (> P21, n=3) were anaesthetized (Ketamine, 100mg/kg) and decapitated. Lumbar dorsal root ganglia were removed and pinned on silicone elastomer blocks before being placed in collagenase (10mg/ml) to soften connective tissue. Ganglia were mounted in a tissue bath, superfused with modified DRG extracellular solution, and patch clamp recordings were made from visualised PVeGFP positive neurons (epifluorescence) using a potassium-based internal solution. **Results:** Neurons expressing PVeGFP had soma diameters of 31 ± 1 µm (n=11), which were significantly larger than adjacent unlabelled neurons (25 ± 1 µm, n=65) in the same DRG. Recordings from 4 PVeGFP positive neurons all showed evidence of hyperpolarization activated current IH. Three of these neurons fired a single AP in response to depolarising current injection and rebound APs following a hyperpolarizing step. **Conclusion:** These results represent the first recordings of identified muscle spindle afferent neurons in the mouse DRG. Our preliminary data suggests that IH is likely to play a role in regulating the excitability of these neurons.

POS-MON-035

PRIMARY AFFERENT NEURON TERMINALS CONTAINING CALCITONIN GENE-RELATED PEPTIDE BUT NOT SUBSTANCE P CONTACT NOXIOUSLY ACTIVATED MURINE DORSAL HORN NEURONS

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Purpose: Many small diameter nociceptive neurons in murine dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). Some mid-sized neurons express CGRP without SP. The targets of these neurons are unknown. Therefore, using the activated extracellular signal-regulated kinase (ERK) as a marker for neuronal activation, we pharmacologically stimulated viable spinal cord slices and determined whether noxiously activated neurons received contacts from terminals containing CGRP without SP using multiple labelling immunohistochemistry. **Methods:** The spinal cord was removed from C57/Bl6 mice and sliced on a vibratome at 300 µm. Spinal cord slices were incubated at 37°C for 3 h before stimulation with 10^{-6}M capsaicin, vehicle, or buffer controls for 10 mins. The slices were subsequently PEG-embedded and sectioned at 20 µm. Activated ERK was detected with phosphorylated ERK (pERK) antibodies on sections also labelled with CGRP and SP. **Results:** CGRP terminals lacking SP were most prominent in lateral areas of lamina I and in lamina IV (n=4 animals). Upon capsaicin stimulation the number of pERK positive cells increased to 34±7 cells compared to 6±2 cells (vehicle control) and 2±1 cells (buffer control) in a single dorsal horn (n=2 animals). Preliminary results show that the pERK containing cells were most prominent in lamina I and II where they received substantial contacts from primary afferent terminals containing CGRP with and without SP (n=2 animals). **Conclusions:** As neurons containing CGRP without SP made contacts on cells noxiously activated by capsaicin it suggests that neurons containing CGRP without SP may have a function in the spinal processing of pain.

POS-MON-036

CHANGES IN FIRING OF SPONTANEOUSLY ACTIVE MUSCLE SPINDLE AFFERENTS DURING TONIC MUSCLE PAIN

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Purpose: The “vicious cycle” theory of chronic muscle pain states that noxious stimulation of muscle causes a fusimotor-driven increase in the firing of muscle spindles, which then increase the excitability of alpha motoneurones to cause a self-sustaining contraction. Intramuscular injection of hypertonic saline in the cat increases mean spindle firing rate by ~80% (Thunberg et al., 2002), but we found no such effect in human subjects (Birznieks et al., 2008). Here we test the hypothesis that long-lasting muscle pain causes a sustained increase in spindle firing. **Methods:** Unitary recordings were made from nine spontaneously active muscle spindle afferents via a tungsten microelectrode inserted into the common peroneal nerve in nine subjects. Tonic muscle pain was induced by intramuscular infusion of hypertonic saline (7%) into the ipsilateral tibialis anterior. All subjects tolerated the pain (5-6/10) for 1 hour. Surface EMG was recorded over the receptor-bearing muscle. **Results:** Mean firing rates did not change significantly during the course of the infusion; measured at 30 mins the mean firing rate was 104.8 ± 4.9% (SE) of the control value. There was no EMG. **Conclusion:** Given that tonic muscle pain caused neither a pronounced increase in firing of muscle spindles, and no increase in EMG, the data do not support the idea that noxious stimulation of muscle causes a self-sustaining contraction via the fusimotor system. Thunberg J, Ljubisavljevic M, Djupsjobacka M & Johansson H (2002). Exp Brain Res 142:319-326 Birznieks I, Burton AR, Macefield VG (2008). J Physiol 586:2713-2723.

POS-MON-037

LONG-TERM IMPACT OF NEONATAL EXPOSURE TO A BACTERIAL MIMETIC ON NOCICEPTION

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Neonatal exposure to the bacterial mimetic, Lipopolysaccharide (LPS), is known to induce a range of permanent psychobiological changes including altered immune, endocrine and behavioural outcomes. Neonatal LPS is also responsible for altered cytokine responses, which play a critical role in the modulation of pain. **Purpose**: To determine the long-term impact of LPS neonatal exposure on nociception using the formalin test. **Methods**: Wistar rats were subjected to either LPS (salmonella enteriditis, 0.05mg/kg, ip) or saline (equivolume) on postnatal days (PND) 3 and 5. At PND13, rats underwent a subcutaneous injection of 0.8% formalin into the plantar surface of the right hindpaw. Subsequent behavioural assessment involved counting flinching and licking the injected paw for one hour post-injection. After behavioural testing, transverse spinal cord slices (300mm thick) were prepared for whole-cell patch-clamp recording (KCH_3SO_4-based internal) from superficial dorsal horn (SDH) neurons. **Results**: Rats subjected to neonatal LPS treatment (n=7) displayed significantly more flinching compared to their matched-control group (n=8) at 5-minutes (p= 0.004) and 15-minutes post formalin injection (p = 0.043). Whole-cell patch-clamp recordings from SDH neurons revealed no differences in input resistance, capacitance or resting membrane potential between rats exposed to LPS and their saline controls (n=18 and 17). The patterns of action potential discharge observed in response to sustained current injection did not differ between groups. **Conclusions**: Neonatal LPS exposure results in elevated behavioural responses to formalin at PND13. This response is not accompanied by changes in selected intrinsic properties of SDH neurons. Ongoing analysis will determine the pathways mediating differences in formalin responses.

POS-MON-038

A NOVEL IN VITRO TECHNIQUE TO RETROGRADELY LABEL DORSAL ROOT GANGLION NEURONS THAT INNERVATE SPECIFIC TARGETS SITES IN VISCERAL ORGANS

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Purpose: Retrograde neuronal tracers are commonly injected into internal organs in vivo to identify spinal afferent neurons in dorsal root ganglia. An unfortunate consequence of injecting neuronal tracers in vivo is that the spread of neuronal tracer is uncontrolled; which means that the identity of the sensory neurons that innervate specific anatomical sites within an organ cannot be determined. To overcome this, we have developed a novel preparation in which bilateral dorsal root ganglia (T13 to L5) were removed from mice, but each DRG retained its neural continuity with the large intestine. Aim: Therefore, our aim was to develop an in vitro technique whereby the identity of specific sensory neurons (in DRG) could be identified that innervate particular anatomical sites in any visceral organ. Methods: In euthanized mice, we extracted bilateral dorsal root ganglia from T13-S5 that retained complete neural continuity with the colorectum. The mucosa and submucosal plexus were sharp dissected away and Cholera Toxin-B was injected into focal sites in the circular muscle layer, 2-4mm from the anus. DRG/colon preparations were incubated in culture for 7 days at 36oC; at which point DRGs were fixed, sectioned and visualized. Results: Fluorescently labeled DRG cells were identified primarily in L6, S1 and S2. A similar proportion of neurons was labeled in each DRG (mean S1 = 30±14 neurons (N=4). Repeating experiments after severing the lumbar colonic and hypogastric nerves in vitro revealed no reduction in labeling (S1 = 52±36 neurons; P>0.05; N=3). Conclusion: This novel technique allows the precise identification of the sensory neurons of spinal afferents that innervate each anatomical layer of the gastrointestinal tract (and other internal organs) to be systematically characterized in vitro, including their morphology and neurochemistry.

POS-MON-039

MODULATION OF NEUROPATHIC PAIN BY REGULATORY T CELLS

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Purpose: Accumulating evidence implicates the immune system, in particular T cells, in the pathogenesis of neuropathic pain. CD4+CD25+Foxp3+ regulatory T cells (Tregs) are endogenous immune suppressors, which reduce T cell proliferation and pro-inflammatory cytokines. Here, we investigated the effect of both expanding and depleting Tregs on pain hypersensitivity in animal models of neuropathy. **Methods**: Following sciatic nerve chronic constriction injury (CCI) or induction of experimental autoimmune neuritis (EAN) rats were treated with superagonistic anti-CD28 antibody (CD28supA, 0.5mg, I.V.) on day 0 and/or day 7 to increase Treg numbers. Following partial sciatic nerve ligation (PSNL) in mice, Tregs were depleted by an anti-CD25 antibody (0.5mg, I.P.). Tregs were analysed by flow cytometry, pain hypersensitivity by sensory tests and immune cell activation (in EAN-affected rats) by immunohistochemistry (n=3-6). **Results**: Treatment with CD28supA on post-CCI days 0 and 7 resulted in recovery of pain hypersensitivity to normal from day 9 onwards ($P<0.05$), which corresponded with a 2-3 fold increase in Tregs in the injured nerve and spinal cord. In EAN-affected rats, CD28SupA treatment on day 7 after disease induction resulted in a significant improvement in pain hypersensitivity on days 13-17 ($P<0.05$). This was associated with significantly fewer T cells and macrophages/microglia in the sciatic nerve, dorsal root ganglia and spinal cord. Finally, treatment of nerve-injured mice with anti-CD25 resulted in a ~70% depletion of CD4+CD25+Foxp3+ Tregs in the lymphoid tissues and enhancement of pain hypersensitivity after PSNL ($P<0.01$). **Conclusion**: Expansion of Tregs decreases, whilst depletion of Tregs increases pain hypersensitivity, suggesting that Tregs play a role in neuropathy-induced chronic pain. Thus, Tregs might be exploited clinically for their potential therapeutic application in neuropathic pain.

POS-MON-040

INTRAVESICAL APPLICATION OF CAPSAICIN AND MUSTARD OIL ACTIVATE DISTINCT NEURAL CIRCUITS IN THE SACRAL SPINAL CORD

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Purpose: Chronic cystitis is a visceral inflammatory state associated with pain and bladder hyperactivity. We aimed to understand the mechanisms underpinning these diverse problems by analysing potential sites of synaptic plasticity in the sacral spinal cord after induction of inflammation and pharmacological activation of bladder nociceptors. **Methods:** In adult female SD rats (n=4 per group), bladder nociceptors were activated by acute intravesical application of transient receptor potential (TRP) channel ligands, capsaicin (TRPV1) or mustard oil (TRPA1). Bladder inflammation was induced by systemic cyclophosphamide (CYP) treatment. To analyse activation of spinal neurons by peptidergic unmyelinated afferents, we performed confocal microscopy of fixed spinal cord sections and counted Neurokinin-1 receptor (NK1R) immunoreactive endosomes in projection neurons within the dorsal horn (DH; lamina 1) and preganglionic neurons in the intermediolateral column (IML). **Results:** NK1R internalisation was observed in both DH and IML neurons after intravesical capsaicin, whereas mustard oil selectively activated NK1R in the IML. Acute (48h) and chronic (10d) cystitis both stimulated NK1R internalisation at each site. Mustard oil enhanced the response of IML neurons to chronic cystitis but had no affect on the DH response. Normal voiding of the bladder (cystometry) caused minimal NK1 internalisation in control animals but a significant increase in the IML of rats with chronic cystitis. **Conclusion:** We propose that TRPA1-expressing bladder afferents preferentially target the micturition reflex pathway whereas TRPV1 afferents activate both autonomic and nociceptive circuits. Understanding the connectivity and cellular changes in the sacral cord may lead to selective pharmacological control of bladder and pain symptoms associated with chronic cystitis.

POS-MON-041

DOES PHOSPHORYLATION OF CAMKII AT MULTIPLE SITES REGULATE CAMKII TARGETING CO-OPERATIVELY OR INDEPENDENTLY?

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Purpose: Calcium/calmodulin-stimulated protein kinase II (CaMKII) is a multifunctional serine/threonine kinase that controls a variety of neuronal processes including synaptic function, learning, memory, ischaemia-induced cell death and cell division. Autophosphorylation of CaMKII at T253, T286 and T305/6 each regulate the binding of CaMKII to specific proteins (targeting) directing CaMKII activity to particular cellular compartments and signalling pathways to produce different functional outcomes. Furthermore, phosphorylation at more than one site can produce different functional outcomes. T286 phosphorylation can induce either LTP or LTD depending on whether T305/6 is also phosphorylated (J Neurosci 30:8704-9). Phosphorylation at T286 or T253 enhances targeting of CaMKII to postsynaptic densities and phosphorylation at both sites gives a cumulative effect (J. Neurochem. 79:1122-8). It is not known whether the functional consequences of phosphorylation at multiple sites are produced by the sum total of independent targeting interactions, each regulated by one phosphorylation site, or by an altered molecular targeting due to a co-operative interaction between multiple phosphorylation sites. **Methods**: Using our overlay binding assay (Cellular Signalling 22:759-69) with protein extracts from different subcellular compartments from brain we have compared the patterns of protein binding obtained with double phospho-mimic mutants of αCaMKII (T253D+T286D, and T286D+T305D) and the corresponding single phosphomimic mutants. **Results & Conclusions**: Preliminary results show that some proteins that did not bind the single phosphomimics did bind the double phosphomimic, while the binding to other proteins was unaffected by the second phosphomimic mutation. This suggests that phosphoryation of CaMKII at multiple sites can alter targeting both independently and co-operatively.

POS-MON-042

SK CHANNELS ENHANCE DENDRITIC EXCITABILITY OF CORTICAL LAYER 5 PYRAMIDAL NEURONS

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Purpose: Dendritic excitability in many neuronal types is mediated by voltage-activated calcium channels (VACCs), leading to large increases in intracellular calcium. How this activity is regulated by calcium-activated potassium channels is poorly understood. **Methods:** To investigate this issue, we made dual whole-cell patch clamp recordings from the soma and apical dendrites of rat cortical layer 5 (L5) pyramidal neurons in brain slices. **Results:** Surprisingly, we observed that blocking dendritic SK channels by local application of apamin to the apical dendrite resulted in a decrease rather than an increase in dendritic excitability, shifting somatic action potential (AP) output during dendritic current injection from bursts to regular firing and increasing the frequency of somatic APs required to generate dendritic calcium spikes (n=10). Since SK channels are co-localized with R-type VACCs in dendritic spines, we investigated the impact of the R-type antagonist SNX482 on dendritic excitability. Local application of SNX482 reduced but did not abolish the occurrence of dendritic calcium spikes and occluded the impact of SK channel block on dendritic excitability (n=8). **Conclusions:** We conclude that SK channels in the apical dendrites of cortical L5 pyramidal neurons enhance rather than reduce dendritic excitability, promoting AP burst firing in response to dendritic input. Furthermore, our results show that this effect is dependent on the activation dendritic R-type VACCs, suggesting that SK channels are co-localized with R-type calcium channels in the dendrites of cortical L5 pyramidal neurons.

POS-MON-043

FUNCTIONAL CHARACTERISATION OF A CHANNELRHODOPSIN-2 TRANSGENIC MOUSE

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Optogenetic methods provide a powerful tool for studying cortical circuitry. As a result, multiple transgenic mouse lines have been developed expressing light-activated channels such as channelrhodopsin-2 (ChR2). A detailed characterisation of these models is vital in determining their limitations and usefulness. **Purpose:** We use a novel light delivery system to investigate functional ChR2 expression in cortical neurons in a ChR2 transgenic mouse. **Methods:** Brain slices were prepared from transgenic mice expressing ChR2 under the control of the Thy1 promoter. Somatic whole-cell recordings were made from cortical layer 2/3 and 5 pyramidal neurons, as well as interneurons, identified by cell morphology, firing pattern and input resistance. Photo-stimulation was achieved using a liquid crystal (LCD) projector system. **Results:** We first assessed which neurons responded to ChR2 photo-activation. Photo-stimulation reliably evoked action potentials in all layer 5 pyramidal neurons examined (n=28). In contrast, photo-stimulation evoked responses in only a subset of layer 2/3 pyramidal neurons (16 out of 24 cells) and interneurons (8 out of 21 cells). To determine the contribution of synaptic input from neighbouring ChR2 expressing neurons to light-evoked responses, we applied antagonists to AMPA, NMDA, and $GABA_A$ receptors. Application of these antagonists did not block light-evoked responses in layer 5 pyramidal neurons, indicating that layer 5 pyramidal neurons are driven by direct ChR2 activation. In contrast, blocking synaptic receptors abolished light-evoked responses in a proportion of layer 2/3 pyramidal neurons (5 out of 12 cells) and interneurons in both layer 2/3 (4 out of 6 cells) and layer 5 (3 out of 5 cells). **Conclusion:** ChR2 expression in the Thy1-ChR2 transgenic mouse shows marked heterogeneity across different cortical layers and between different cell types.

POS-MON-044

WHAT CHANNELS UNDERLIE GABA-B MEDIATED IPSPS IN CORTICAL PYRAMIDAL NEURONS?

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GABA is the primary inhibitory neurotransmitter in the brain, where it acts at either GABA-A (ionotropic) or GABA-B (metabotropic) receptors. In many brain regions activation of GABA-B receptors generates a slow IPSP, thought to be mediated by the opening of G protein-coupled inwardly rectifying potassium channels (GIRK channels). Whether this is the case or not in cortical pyramidal neurons has never been directly examined. **Purpose**: To determine the channels activated by GABA-B receptors in pyramidal neurons in the neocortex. **Methods**: Somatic whole-cell recordings were made from cortical layer 5 or hippocampal CA1 pyramidal neurons in brain slices from 8-week-old rats. GABA-B responses were evoked by local somatic application of the GABA-B receptor agonist baclofen (50 μM). **Results**: We first examined the impact of the GIRK channel blocker tertiapin-Q (100 nM) on GABA-B responses under voltage-clamp. Surprisingly, bath application of tertiapin-Q did not affect GABA-B responses in cortical pyramidal neurons ($P>0.05$, n=4), despite reducing GABA-B responses in hippocampal pyramidal neurons. In contrast, GABA-B responses in cortical pyramidal neurons were decreased by bath application of the two-pore domain "TASK" potassium channel blocker bupivacaine (40 μM; $P<0.001$, n=5), which also led to a significant increase in somatic input resistance and depolarisation of the resting membrane potential ($P<0.05$, n=4). **Conclusions**: These data suggest that GABA-B mediated slow inhibition in cortical pyramidal neurons is not mediated via GIRK channels, as classically described in other brain areas. In contrast, we provide evidence that in cortical pyramidal neurons GABA-B mediated responses activate a TASK-like potassium conductance. Further experiments will be required to determine the channels activated by synaptic GABA-B receptors in these neurons.

POS-MON-045

PERIPHERAL TARGETS OF 5-HT1D RECEPTOR IMMUNOREACTIVE TRIGEMINAL GANGLION NEURONS

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Purpose: $5HT_{1D}$ receptors have been reported on peripheral terminals of trigemino-vascular neurons. Activation of these receptors with triptans ($5HT_{1D}$ receptor agonists) is thought to inhibit calcitonin gene-related peptide (CGRP) release, and result in decreased vasodilation of intracranial vasculature and reduced pain in migraine. However, the proportion of trigeminal primary afferent neurons that innervate the intracranial vasculature and other craniofacial tissues, that are also immunoreactive for $5HT_{1D}$ receptors, is unknown. **Methods:** Retrograde tracing and immunohistochemistry was used to identify $5HT_{1D}$ receptor labelled trigeminal primary afferent neurons that innervate the lacrimal gland (n=3 animals), nasal mucosa (n=3 animals), and the intracranial vasculature (middle meningeal artery in the dura (MMA; n=3 animals) and middle cerebral artery (MCA; n=3 animals)). **Results:** The percentage of $5HT_{1D}$ receptor immunoreactive trigeminal primary afferent neurons that innervate the MMA (41.8 ± 1%) was greater than the percentage that innervates the MCA (28.4 ± 0.8%), nasal mucosa (25.6 ± 1%) or lacrimal gland (23.5 ± 3%). For each retrograde labeled population, the $5HT_{1D}$ receptor immunoreactive cells were amongst the smallest of the retrograde labelled cells. **Conclusion:** These findings provide a basis for understanding the role of $5HT_{1D}$ receptor agonists (e.g. triptans) in the treatment of primary vascular headaches and suggest that the selectivity of triptans in the treatment of these headaches does not appear to result from specific localization of the $5HT_{1D}$ receptor to trigeminovascular neurons alone.

POS-MON-046

MIDBRAIN PROJECTIONS TO THE DORSAL LATERAL GENICULATE NUCLEUS IN MARMOSET

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Purpose: To characterise extra-retinal, non-cortical inputs to the lateral geniculate nucleus (LGN) in marmosets (*Callithrix jacchus*). **Methods:** Iontophoretic injections of a tetromethylrhodamine and biotinylated dextran amine conjugate (Microruby) were made in the left LGN of 4 sufentanil-anaesthetised, adult marmosets. Injections were targeted at parvocellular (PC, 2 animals), magnocellular (MC, 1 animal) or koniocellular (KC, 1 animal) layers of the LGN. Retrograde transport of the tracer was allowed for 50 - 90 hours following injection. The animals were overdosed with sodium pentobarbitone (300 - 600 mg/kg), perfused with paraformaldehyde, and the brains were removed. The brainstem was sectioned coronally into 50 µm sections. Every third section was processed using diaminobenzidine tetrahydrochloride (DAB) - horseradish peroxidase reaction. **Results:** In all 4 marmosets, the ipsilateral (but not the contralateral) superior colliculi and the parabigeminal nuclei contained retrogradely labelled cells. In three marmosets the ipsilateral pretectal nucleus of the optic tract also contained retrogradely labelled cells. The density of retrograde labelling in the parabigeminal nuclei was highest in the 2 marmosets in which the injections were largely restricted to the parvocellular layers of the LGN. **Conclusions:** 3 regions of the marmoset midbrain have been shown to send ipsilateral projections to the LGN. The lack of clear segregation of projections from parabigeminal nucleus to distinct (PC, MC, KC) subdivisions of the LGN, suggests that this cholinergic projection might influence all three cellular components of ipsilateral LGN fairly uniformly.

POS-MON-047

WHOLE CELL CONDUCTANCES OF DEVELOPING HUMAN HAIR CELLS

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Background: The majority of studies investigating the development of peripheral vestibular function have focused on animal models. Here we report a timeline of functional development in human vestibular hair cells and afferent terminals during a period of significant change when whole cell conductances begin to mature. **Methods:** Human tissue was collected according to regulatory requirements of the University of Newcastle Human Research Ethics Committee. Inner ears from electively terminated human foetuses (11 to 18 weeks gestation; WG) were isolated and semicircular canal cristae excised in ice-cold glycerol-based Ringers' solution. Tissue was transferred to a recording chamber perfused with oxygenated L15 cell culture medium. Whole-cell patch-clamp recordings using potassium fluoride internal solution were made from embedded hair cells and calyx terminals. **Results:** We have recorded and intracellularly labelled human hair cells that display inward and outward rectifying conductances (n=47). During early fetal gestation (11-13 WG), some immature hair cells (n=3) show evidence of sodium conductances. The earliest expression of the mature type I hair cell conductance, $g_{K,I}$, was observed at 14 WG. This approximately coincides with our first recordings from calyx afferent terminals (15 WG). Putative, type II hair cells have a mean peak amplitude of 1.2 nA (n = 7; 11-13 WG) that increases to 2.7 nA (n = 11; 17 -18 WG). **Conclusion:** Our data show human hair cells aged 11-13 WG are still functionally immature. By 14 and 16 WG, hair cells begin to express mature conductances, including those typically seen either in mature type I or type II hair cells. In addition, there is a concomitant maturation of calyceal terminals contacting putative type I hair cells.

POS-MON-048

GENES AND NEURAL CIRCUITS CONTROLLING SENSITIVITY TO CARBON DIOXIDE IN THE MOSQUITO

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Purpose: Blood-feeding mosquitoes, such as the yellow fever mosquito *Aedes aegypti*, use highly specialized and sensitive olfactory systems to locate their hosts. This sensory process involves detecting and following plumes of volatile host emissions, which include skin odor and carbon dioxide (CO_2). In mosquitoes, CO_2 functions as a powerful attractant and additionally acts to sensitize the mosquito olfactory system, alterting them to the presence of human skin odor and heat. Despite the critical importance of CO_2 reception in driving the orientation of mosquitoes towards humans, the molecular and cellular basis of how this gas is sensed and integrated with other host chemical and physical cues to initiate host-seeking behavior is poorly understood. **Methods:** As a first step towards dissecting CO_2 sensation in the mosquito, we used zinc-finger nuclease (ZFN) technology to generate null mutations in two gustatory receptors, *AaGr1* and *AaGr3*, that putatively function as CO_2 receptors in *A. aegypti*. We further characterized the electrophysiological and behavioral responses of these mutant mosquitoes to CO_2. **Results:** Non-sense mutations were successfully recovered for both *AaGr1* and *AaGr3* using ZFN-mediated targeted mutagenesis and sib-selection. *AaGr3* null mutants were selected for initial characterization, and were found to be electrophysiologically unresponsive to CO_2. Furthermore, in a behavioral paradigm of CO_2-induced heat seeking, *AaGr3* mutants were behaviorally insensitive to CO_2, when compared to wild-type mosquitoes ($P < 0.001$). **Conclusion:** Our data provides compelling evidence that *AaGr3* is required for CO_2 sensation in *A. aegypti*, and suggests that CO_2 sensation gates the response of female mosquitoes to heat.

POS-MON-049

RETINAL GANGLION CELL THRESHOLDS TO ELECTRICAL STIMULATION USING HEXAGONAL GUARD RETURN AND MONOPOLAR RETURN CONFIGURATIONS

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Purpose: Retinal visual prostheses rely on electrically stimulating retinal ganglion cells (RGCs) via electrodes implanted in or near the retina, resulting in percepts of light termed 'phosphenes'. Activation of spatially distinct populations of RGCs requires electrical stimulation to be localised to the area in the direct vicinity of the stimulating electrode(s). The hexagonal guard return (hex return) configuration has been proposed as an alternative to the traditional monopolar return configuration in order to achieve localised retinal activation. The hex configuration utilizes six electrodes surrounding a central stimulating electrode, with the hex acting as a combined return. In this study, the efficacy of the hex return configuration to localise charge was investigated in isolated whole-mount rabbit retina. **Methods:** Electrical stimulation was applied via a Multielectrode Array (MEA), located subretinally, in both monopolar and hex return configurations. Whole-cell patch clamping was used to record the activation threshold of RGCs (n=22) in the rabbit retina in response to electrical stimulation using both hex and monopolar return configurations. **Results:** No statistical difference in RGC activation thresholds was found between hex and monopolar return configurations for cells located inside the hex. RGCs located outside the hex displayed activation thresholds that were significantly higher (>2 fold) for the hex return compared to the monopolar return configuration ($p<0.01$). **Conclusion:** The hex return configuration localised charge associated with electrical stimulation more effectively than monopolar return configurations. This may provide an improved method of electrical stimulation of neural tissue where there is a requirement to activate spatially distinct populations of cells.

POS-MON-050

NEUROBIOLOGICAL CONSEQUENCES OF ACUTE FOOTSHOCK STRESS

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Stress is a major burden on our society. The aim was to investigate the effects of acute footshock stress on the hypothalamo–pituitary–adrenocortical (HPA) axis and the adrenomedullary catecholaminergic system. **Methods:** Sham and footshock rats were placed into programmable footshock chamber and did not receive or received footshock (1mA, 1sec pulse per min) for 10min, 20min or 40min (n=6/group). Animals were sacrificed after the experimental protocols. Plasma samples were analysed by radioimminoassay for adrenocorticotropic hormone (ACTH) and corticosterone and by glucose-meter for glucose. Adrenal medulla (AM) samples were analysed by immunoblotting for tyrosine hydroxylase (TH) protein and phosphorylation and by tritiated-water-release assay for TH activity. **Results:** Footshock caused a significant increase in ACTH at 20min (5.245 fold, $p<0.001$), corticosterone at 20min (1.414 fold, $p<0.05$) and 40min (1.525 fold, $p<0.05$) and glucose at 20min (1.550 fold, $p<0.001$) and 40min (1.158 fold, $p<0.05$). TH protein and pTH-Ser19 were not significantly altered at 10min, 20min and 40min. Footshock caused a significant increase in pTH-Ser31 at 10min (1.687 fold, $p<0.01$), 20min (1.482 fold, $p<0.05$) and 40min (1.521 fold, $p<0.05$) and pTH-Ser40 at 40min (1.473 fold, $p<0.01$). Footshock caused a significant increase in TH activity levels at 20min (2.117 fold, $p<0.01$) and 40min (2.803 fold, $p<0.001$). **Conclusion:** These findings indicate that acute footshock led to 1) HPA axis activation which involved the release of ACTH and corticosterone and an increase in plasma glucose followed a pattern similar to corticosterone; 2) adrenomedullary catecholaminergic system activation which involved TH phosphorylation, primarily at pTH-Ser31 and pTH-Ser40 and an increase in TH activity levels.

POS-MON-051

ACTIVATION OF SPINALLY PROJECTING OREXIN NEURONS DURING NOVELTY, FEAR AND EXERCISE

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Purpose: Recent work shows that orexin contributes to the cardiovascular response of novelty and fear. Orexin neurons project to premotor sympathetic centres in the brainstem, but are also premotor themselves since they can directly target sympathetic preganglionic neurons in the thoracic cord. The aim was to determine how much of these orexinergic neurons activated by novelty, fear and exercise are spinally projecting. **Methods**: Bilateral injections of the retrograde tracer Cholera toxin subunit B (CTB) were made in the thoracic cord (T2-T3). Two weeks later, the rats were tested (40 min) for: contextual Fear, Novelty (new box) and Exercise (treadmill 10 m/min), then perfused. Control was Rest. Sections of the hypothalamus were analysed for triple immunofluorescence labelling of CTB, orexin and Fos. **Results**: Approximately 11% of orexin neurons were CTB labelled. Fear, Novelty and Exercise increased the proportion of orexin neurons expressing Fos from 5% at Rest to 26, 42 and 39% respectively ($p<0.001$), however the proportion of Fos expressing orexin neurons labelled with CTB did not change across test groups (9, 12 and 16%, $p=0.06$). Approximately 15% of CTB labelled neurons contained orexin. Fear, Novelty and Exercise increased the proportion of CTB labelled neurons expressing Fos from 13% at Rest to 17, 22 and 21% respectively ($p<0.03$). This increased Fos in CTB labelled neurons was due in large part to those that contained orexin (from 10% at rest to 23, 54 and 45% respectively). **Conclusion**: At least 10-15% of orexin neurons activated by Novelty, Fear and Exercise project as far down as the thoracic cord. These long premotor orexinergic projections could make a significant contribution to the cardiovascular response of arousal, stress and exercise.

POS-MON-052

BISTABLE PERCEPTION MODULATED BY CONDITIONED FEAR FOR INVISIBLE STIMULI

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Background: Previously, we showed that perceptual awareness during bistable perception is affected by fear conditioning (Kim, Lee, Kang & Kim, 2010). Specifically, a visual interpretation associated with an aversive electrical stimulation (CS+) tended to predominate over another visual interpretation not associated with stimulation (CS-) for observers who showed heart rate modulation during conditioning. In the current study, we went further to investigate whether such changes in bistable perception can also be caused by invisibly conditioned fear. Methods: The current study consists of 1) pre-conditioning bistability test 2) unconscious fear conditioning, and 3) post-conditioning bistability test. During the pre- and post-conditioning bistability test, observers tracked their perceptual experiences for man-rat and duck-rabbit ambiguous figures. During unconscious fear conditioning, two unambiguous variants of the man-rat figure were used as conditioned stimuli (CS) and presented to the suppressed eye during continuous flash suppression (CFS, Tsuchiya & Koch, 2005). One of the two variant images (CS+) was paired with electrical stimulation on a finger (US) while the other image (CS-) was not. Observers' heart rate was monitored during unconscious fear conditioning. Results: We found that CS+ predominated over the other CS- even after unconscious fear conditioning. Such change was found in observers who showed heart rate difference to CS+ and CS- and who also showed high state-anxiety scores (n=10). Conclusion: Visual awareness during bistable perception is influenced by invisibly conditioned fear, especially in highly anxious observers. This research is supported by Ministry of Culture, Sports and Tourism(MCST) and Korea Creative Content Agency(KOCCA) in the Culture Technology(CT) Research & Development Program 2011

POS-MON-053

DOPAMINE ADMINISTRATION INTO THE RAT SUBTHALAMIC NUCLEUS PRODUCES A CONDITIONED PLACE PREFERENCE THAT IS PREVENTED BY THE CO-ADMINISTRATION OF OXYTOCIN

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Purpose: Methamphetamine is an illict drug of abuse which produces reward through enhanced dopamine release. We have recently shown that the administration of the neuropeptide oxytocin attenuated the rewarding effects of methamphetamine at the level of the subthalamic nucleus (STh). Oxytocin is thought to reduce methamphetamine-related reward through modulating dopamine activity and release, however little is known about the rewarding effect of dopamine at the STh or the interaction that oxytocin may have on such an effect. Our aims were twofold: i) to determine if a microinjection of dopamine in the STh would result in a single-trial conditioned place preference (CPP) for dopamine and ii) to investigate the effect of the co-administration of oxytocin and dopamine into the STh on dopamine CPP. **Methods:** Male Sprague-Dawley rats (n=32) underwent surgery for the implantation of bilateral microinjection cannulae (26 GA) in the STh under isoflourane anaesthesia. After 5 -7 days of recovery, CPP was conducted using 2 daily conditioning sessions (30 min), where treatment (dopamine (100nmol/side) or dopamine + oxytocin (100nmol/side + 0.6 pmol/side)) was paired with 1 context, and vehicle administration was paired with the other context. **Results:** Our results showed that a single trial dopamine CPP increased the time rats spent in the dopamine-paired context and decreased the time spent in the vehicle-paired context across testing days, indicating a CPP for dopamine ($p < .05$). A single trial CPP with a co-administration of dopamine and oxytocin prevented the formation of a CPP for dopamine ($p = .459$). **Conclusion:** These findings show that dopamine administration into the STh produces a rewarding effect that is reduced by the co-administration of oxytocin.

POS-MON-054

EXCITOTOXIC LESIONS OF THE AMYGDALA DISRUPT SIGN-TRACKING BUT NOT GOAL-TRACKING IN RATS

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Purpose: Sign-tracking, or autoshaping, describes the process by which an animal learns that a cue (CS) predicts a particular outcome (US) and comes to respond to that cue as though it is the outcome itself (e.g. approaching and biting at a light that predicts food). Previous research has implicated the central nucleus of the amygdala (CeA) in the acquisition of this behaviour, though some studies have also suggested that this region may be involved in the acquisition of conditioned approach behaviour in general. In order to address this discrepancy, the current research aimed to explicitly examine the role of the CeA in the acquisition of sign-tracking (i.e. approach to the CS) compared to goal-tracking (approach to the US) behaviour. **Methods:** Rats were given excitotoxic lesions of the CeA ($n = 6$) or sham surgery ($n = 8$), and were subsequently trained on a Pavlovian conditioning procedure where 10s presentations of a lever were followed by delivery of individual food pellet rewards. Training sessions consisted of 25 lever-pellet pairings, and animals received 2 sessions per day over 10 days. Engagement with the lever (biting, grasping etc.) and entries into the food magazine were recorded as measures of CS and US approach respectively. **Results:** Results indicate that CeA lesions significantly disrupt sign-tracking behaviour ($F_{9,108} = 3.041$, $p < .05$), but have no effect on goal-tracking behaviour ($F < 1$). **Conclusion:** This study supports previous findings that the CeA is important for development of sign-tracking responding. In addition, results confirm that the involvement of this region is selective to sign-tracking and does not generalize to other conditioned approach responses (i.e. goal-tracking).

POS-MON-055

THE EFFECT OF INHIBITING DNA METHYLATION IN THE RAT DORSAL HIPPOCAMPUS ON LONG-TERM RECOGNITION MEMORY

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Purpose: A deeper understanding of the biological mechanisms that underlie memory formation is urgently required to enable the development of treatments for various memory disorders. Recent research has suggested that DNA methylation is involved in the consolidation of long-term memory, and inhibition of the enzymes which catalyse DNA methylation, DNA methyltransferases (DNMTs), prevents the establishment of long-term fear memory. The current study aimed to determine the effect of DNMT inhibition on object recognition memory. **Methods:** A pilot study was conducted to verify that the novel object recognition test was able to measure recognition memory in male Sprague Dawley rats (n=24). For the main experiment, male Sprague Dawley rats (n=64) underwent intracranial surgery for bilateral insertion of cannulae into area CA1 of the hippocampus (coordinates AP: -3.6mm relative to bregma; ML: ± 1.7mm; DV -2.6mm from skull) under isoflurane anaesthesia. After recovery, rats were exposed to a single object-learning sample, followed immediately by microinjection of either the DNMT inhibitor zebularine (.5µl) or vehicle dimethyl sulfoxide (2 % DMSO) (.5µl). Recognition memory was assessed at 24 or 48 hours after sample (n=32 per time-point). **Results:** The pilot study indicated the object recognition task was viable (p=.07). In the main experiment at 24-hour test, zebularine treated rats did not display recognition memory, while the vehicle group did recognise the familiar object ($F(1)=5.07$, $p<0.05$). Conversely, at the 48-hour test, both zebularine and vehicle treated rats demonstrated recognition memory ($F(1)=5.21$, $p<0.05$). **Conclusion:** The present findings suggest the DNMT inhibition in the CA1 region of the hippocampus disrupts recognition memory in a time-dependent manner.

POS-MON-056

THE NEURAL BASIS OF THE PARTIAL REINFORCEMENT EXINTINCTION EFFECT

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Introduction: In Pavlovian and instrumental conditioning, omission of unconditional stimuli from a proportion (e.g. 50%) of trials in a continuous (100%) learning schedule generally produces increased resistance to extinction of conditioned responses, a paradoxical phenomenon known as the partial reinforcement extinction effect (PREE). The neural mechanisms underlying the PREE remain unclear. **Methods**: 10 week old male Wistar rats (n=12) were subjects in an aversive Pavlovian (fear) conditioning experiment employing a 20 second 90dB 5000Hz tone as the conditioned stimulus (CS) and a coterminating 0.5 second 0.6 mA foot shock as the unconditioned stimulus (US). Subjects were divided into four experimental groups each receiving a different probability of reinforced trials during acquisition of conditioning: 0.25, 0.5, 0.7 and 1.0. Freezing behaviour was used to index fear learning. The animals were sacrificed after extinction and 50µm coronal sections of their brains were labelled with antibodies for p42/44 MAPK (Erk1/2) and c-fos. **Results**: In extinction, all three partial groups showed increased levels of freezing (0.25:6.66%, 0.5:42.06%, 0.7:13.75%) compared with the continuous group (1.0:6.25%). The 0.5 group freezing was significantly greater ($p<0.001$) than the 1.0 group. Increased p-erk labelling (mean number of cells per section) was observed in central amygdala in the partial groups (0.25:12.17, 0.5:22.39, 0.7:14.44) compared to the continuous group (1.0:3.65). Greater increases in p-erk and c-fos labelling was also observed in basolateral amygdala, hippocampus, prelimbic and infralimbic cortex of partially reinforced animals compared to continuous. **Conclusion** Resistance to extinction following partial reinforcement displayed an inverted U function with respect to reinforcement probability. Neural activity in amygdala, hippocampus and infralimbic and prelimbic cortices showed a similar inverted U relationship to reinforcement probability. These results are consistent with a Bayesian interpretation of condidtioning and the PREE, in which uncertainty or entropy plays a critical role.

POS-MON-057

NEURAL ACTIVITY DYNAMICS DURING ACQUISITION OF LEARNT FEAR IN AN AUDITORY CUED CONDITIONING PARADIGM

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Purpose: The acquisition of new memories, i.e. learning, is supported by long lasting changes in neuronal activity in networks of neurons spanning multiple brain regions. Understanding how these memories are formed, stored and retrieved requires tracking the activity of neural assemblies during these different phases with single neuron resolution. We used electrophysiology in awake behaving animals to record neuronal activity in different brain regions during the successive phase of associative learning. **Methods:** We used multi wire electrodes (8 tetrodes) mounted in independently moveable drives to target the regions of interest. A week after implantation the rats (n=4) were introduced to the conditioning environment and the electrode were lowered into the amygdala and subiculum. The conditioning protocol consisted of 20 sec tones paired with a footshock (0.6mA, 0.5s, 7 pairings) and the animals were extinguished during two separate sessions (28 presentation each day). **Results:** Multi wire recording were analyzed to separate individual units and follow their activity across multiple recording sessions using waveform features. We compared changes in neuronal activity within the different phases of learning, test and extinction sessions and tested these changes against the behavior observed. **Conclusion:** We show that different neurons in a given region do not behave identically during the acquisition or recall of particular memories. Moreover, the dynamics of activity appear to be different between different groups of neurons.

POS-MON-058

AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE NUCLEUS ACCUMBENS IN RESPONSE TO REWARD

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Purpose: The Nucleus Accumbens (NAc) is a significant neural substrate of the brains reward system, and is thought to play a critical role during goal-directed behaviours for natural reinforcement and drug addiction. The aim of this study was to identify how neuronal firing patterns within the NAc correlate specifically with the acquisition of ethanol during a cue-induced model of relapse. Furthermore we aim to elucidate if electrical stimulation of this region can reverse a preference for ethanol as well as self-administration of the drug. **Methods:** Six alcohol preferring (iP) rats were anesthetized and surgically implanted with a 32-channel electrode into the NAc, using stereotaxic coordinates. Post recovery, a wireless headstage was attached and animals underwent testing in an operant chamber. Animals were allowed to self-administer either ethanol or water by pressing a lever. Neural activity was recorded during this paradigm using TDT equipment. Once responses for ethanol were stable rats underwent similar testing whereby they could either self-administer water paired with electrical stimulation of the NAc, or water with no stimulation. **Results:** Neural recording from implanted animals displayed anticipatory increases in firing rate within the NAc in response to self-administering ethanol. Furthermore, during electrical stimulation of the NAc, rats showed a significant preference for stimulation vs. non-stimulation paired levers (p=0.0034), similar to that of ethanol vs. water (p<0.0001). **Conclusion:** Electrophysiological recordings within the NAc demonstrated neural activation specific to the self-administration of ethanol. When electrical stimulation was administered, the animal displayed a preference for stimulation that was comparable to ethanol, therefore suggesting that electrical stimulation of the NAC could potentially override the motivation to self-administer ethanol and reduce vulnerability to relapse.

POS-MON-059

GTF2IRD1, IMPLICATED IN THE WILLIAMS SYNDROME COGNITIVE PROFILE, IS INVOLVED IN CHROMATIN REGULATION

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Williams-Beuren syndrome (WBS) results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. Its features typically involve a set of cognitive and behavioural symptoms that are consistently present irrespective of social or ethnic background, thus providing compelling evidence of a genetic basis for aspects of human cognition and behaviour. Recent human mapping data implicates a transcriptional regulator, encoded by the *GTF2IRD1* gene, as a principal player in the origin of these neurological defects. We generated *Gtf2ird1* knockout mouse lines that show some striking similarities to aspects of the human disease. **Purpose**: To understand the cellular and molecular basis of these defects, we have conducted detailed studies of *Gtf2ird1* gene expression and analysed the biochemical properties of the GTF2IRD1 protein. **Results**: Post translational modifications regulate GTF2IRD1 stability while transcript levels are tightly regulated by direct auto-repression. These data suggest that cells are highly sensitive to changes in GTF2IRD1 levels, thus explaining why haploinsufficiency of this gene leads to features of WBS. We have demonstrated that GTF2IRD1 is regulated via SUMOylation through substrate-specific interactions with PIASxβ. GTF2IRD1 also interacts with histone deacetylase proteins via binding with ZMYM5 and binds to a novel histone methyltransferase, SETD6. **Conclusion**: Our work positions GTF2IRD1 as an important new player in the epigenetic regulation of chromatin structure in the brain and promises insights into how such mechanisms can impact on the development of neuropsychiatric disorders.

POS-MON-060

ENHANCED HYPERLOCOMOTION IN RESPONSE TO NMDA RECEPTOR BLOCKADE IN MALE REELIN HETEROZYGOUS MICE IS REVERSED BY GONADECTOMY: COMPARISON WITH NMDA RECEPTOR DENSITY

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Purpose: Schizophrenia is a severe and debilitating mental illness most likely caused by early genetic or environmental changes in neurodevelopment. Reelin is involved in cortical development and its levels are significantly reduced in schizophrenia. **Methods:** Hyperlocomotion induced by treatment with the NMDA receptor antagonist, MK-801, was used in Reelin heterozygous ($Rln^{+/-}$) and wildtype control mice (n=10-18 per group) as a model of psychosis. Forebrain NMDA receptor density was measured with [^{3}H]MK-801 autoradiography. **Results:** The effect of MK-801 was greater in male mice than in female mice and significantly enhanced in male, but not female $Rln^{+/-}$ mice compared to sex-matched controls. Gonadectomy (by surgical castration) reduced MK-801-induced hyperlocomotion in male mice to the level seen in females and abolished the difference between the genotypes. In contrast, there was no effect of ovariectomy in female mice. Forebrain NMDA receptor density was slightly, but significantly increased by castration in male mice, particularly in the caudate nucleus. In contrast, ovariectomy slightly decreased NMDA receptor density in female mice, particularly in the caudate nucleus, motor cortex and prelimbic cortex. None of these changes in binding density were different between $Rln^{+/-}$ mice and wildtype controls. **Conclusion:** These results suggest that male sex hormones enhance the effect of NMDA receptor blockade on behaviour and that this effect is greater in Reelin deficiency. This interaction was not seen in female mice and was independent of NMDA receptor density, at least in the forebrain regions analyzed. These results could increase our understanding of sex differences in the role of Reelin deficiency in schizophrenia.

POS-MON-061

CONSTRUCTION OF EGFP HSV-1 H129, A NOVEL HERPES VIRAL TOOL TO STUDY THE ORGANISATION OF NEURAL CIRCUITS

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Neurotropic viruses are widely used to define the organisation of neural circuits. The H129 strain of HSV-1 uniquely and selectively travels in the anterograde direction along synaptically connected neurons. **Purpose:** To construct recombinant H129 virus that expresses an enhanced green fluorescent reporter protein. **Methods:** A CMV driven EGFP expression cassette was inserted via homologous recombination into the intergenic region between the UL26/26.5 and UL27 genes within the H129 genome. EGFP-H129 virus was plaque purified before being characterised in vitro and compared with the wildtype-H129 virus using in vivo airway sensory neuron circuit tracing. **Results:** Purified EGFP-H129 viral stocks contained no detectable wildtype-H129. The EGFP-H129 virus replicated as efficiently as wildtype-H129 in single cycle growth experiments. 72 hours post-inoculation of the rat extrathoracic trachea (n=4) with $7x10^7$ pfu/ml, strong EGFP fluorescence was evident in a subset of vagal sensory neurons. Brainstems from animals infected with either wildtype or EGFP-H129 (n=12) 72-96 hours earlier contained viable virus particles, albeit the titre of wildtype-H129 (148.6±71.9 pfu/gm) was slightly higher than EGFP-H129 (92.3±50.0 pfu/gm). Both viruses were localised to neurons in the medial nuclei tractii solitarii and spinal trigeminal nucleus. By 120-144 hours post-inoculation, wildtype-H129 was present in the thalamus, subthalamus, amygdala, primary somatosensory and insular cortices. EGFP-H129 was detectable in comparable thalamic, subthalamic and amygdala regions, but had not yet reached cortical loci. **Conclusion:** Insertion of the EGFP cassette into the H129 genome had no significant effect on growth, replication and anterograde transneuronal motility properties. EGFP-H129 will be a valuable tool for studying neuronal circuitry in vivo.

POS-MON-062

THE EFFECT OF EXERCISE ON SOCIALITY, ANXIETY, LOCOMOTOR ACTIVITY AND HIPPOCAMPAL NEURONS IN RATS

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Exercise, while improving cardio-respiratory fitness, has also been demonstrated to have beneficial central effects such as cognitive enhancement, improvement of quality of life and neuroprotection. The aim of the present study was to assess the effect of treadmill running on mood and locomotor behavioural measures and hippocampal GABAergic neuronal number and brain-derived neurotrophic factor (BDNF) levels. Male Long-Evans rats underwent a low intensity exercise regimen consisting of running on a treadmill at 10metres/min, 30 min/day, for 5 weeks (EX); while control animals received daily handling (CON). Social interaction and anxiety behaviour and basal locomotor activity were assessed at the end of the training period. Following on from this hippocampal BDNF measures and parvalbumin immunohistochemistry was performed. While low intensity exercise had no effect on basal locomotor or anxiety levels, EX animals interacted more with an unknown rat than CON animals (no interaction scores ($p<0.01$)). On analysis of the hippocampus, increased numbers of parvalbumin-immunoreactive neurons were observed in all areas of the hippocampus (DG ($p<0.01$); CA1 ($p<0.01$); CA2/3 ($p<0.05$)); while BDNF levels remained unchanged. These results demonstrate that low intensity exercise has positive effects on 'mood' measures and hippocampal pathology and suggests a role for it as a positive intervention in models of psychosis and diminished mood.

POS-MON-063

AGE AND AGENT DEPENDENT EFFECTS OF VOLATILE ANAESTHETICS ON COGNITION POST-EXPOSURE IN RATS

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Purpose: Postoperative cognitive dysfunction occurs in ~10% of all patients 1 week post-surgery with higher incidence and more prolonged dysfunction in the elderly. Isoflurane exposure in young and aged rodents causes delayed neurocognitive deficits. Sevoflurane and desflurane are reported to induce apoptosis and exacerbate Alzheimer's pathology early after exposure (Dong YL et al 2009 Arch Neurol.; Zhang B et al 2008 J Biol Chem) but their effects on cognition have not been tested. Methods: Young adult (3 mo; n=40) and aged (18-20 mo; n=33) male Sprague Dawley rats were exposed to sevoflurane or desflurane (1 MAC, 4h) or control conditions. Learning (acquisition phase) and memory (probe trial) were tested in the water maze 1, 4 and 12 weeks post-exposure. Latency to locate the hidden platform, and time spent in the platform quadrant were compared between ages and treatments. Results: Desflurane exposure significantly impaired acquisition of the water maze task 1 week after exposure in aged but not young rats. This impairment was no longer apparent at 4 or 12 weeks. In contrast, sevoflurane significantly enhanced acquisition of the water maze task in both young and aged rats. This enhanced performance was maintained at 4 and 12 weeks in young but not in aged rats. Neither sevoflurane nor desflurane had any effect on memory for platform location compared with controls in any of the probe trials. Conclusion: Sevoflurane and desflurane have delayed effects on cognition and their effect is age and agent dependent. Early pathological changes after anaesthetic exposure may be unrelated to neurocognitive outcome. Sevoflurane may be the anaesthetic of choice for elderly patients.

POS-MON-064

ADOLESCENT TOLUENE INHALATION: IMPLICATIONS FOR ETHANOL CONSUMPTION AND LIVER FUNCTION

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Purpose: Exposure to substances of abuse often occurs during adolescence, with inhalation of volatile organic solvents such as toluene commonly being the initial drug experienced. Adolescent abuse of such inhalants poses a significant risk to the maturing brain significantly increasing the chance of subsequent drug use and dependence in adulthood. **Methods:** Two cohorts of adolescent (PND 30 upon commencement) male Wistar rats were exposed to either air (n=12 per cohort) or chronic intermittent toluene (CIT, 3000 ppm, n=12 per cohort) 1hr per day, 3 days per week; an exposure pattern recapitulating that seen in the human situation. Following either 4 or 8 weeks exposure, we investigated the voluntary consumption of 20% ethanol in a chronic intermittent two-bottle free choice paradigm for up to 10 weeks. Hepatic ethanol metabolism and liver pathology were also assessed. **Results:** Ethanol consumption increased over time ($p<0.001$) following both 4 and 8 weeks exposure to either air or CIT. However, no differences in ethanol or total fluid consumption were observed between air and toluene exposed rats. Also, there were no differences in consumption between groups following 7 days of ethanol deprivation. These results were not confounded by non-specific effects upon liver metabolism or histopathology. **Conclusions:** Adolescent toluene inhalation for 4 or 8 weeks under the current paradigm had no impact on voluntary consumption of 20% ethanol in adulthood in rats. However, the implications of adolescent toluene inhalation upon other aspects of drug-seeking behaviour are yet to be determined.

POS-MON-065

THE INHIBITION OF HISTONE DEACETYLASES DURING EXTINCTION OF NICOTINE SELF-ADMINISTRATION ATTENUATES BOTH CUE- AND NICOTINE-INDUCED REINSTATEMENT

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Purpose: Recent research has implicated central epigenetic mechanisms in the establishment and maintenance of drug-seeking behaviour. The aim of the present study was to examine the influence of histone deacetylase (HDAC) inhibition on the extinction and reinstatement of nicotine-self administration. **Method:** Male Sprague Dawley rats first underwent surgery for the implantation of chronic intravenous catheters. Following this, rats were trained to acquire nicotine self-administration under an FR-1 schedule of reinforcement for a total of 12 days. Under these conditions, each response on the active nose-poke resulted in a single infusion of nicotine (30 µg/kg/100µL), and presentation of a cue-light. Responding for nicotine was then extinguished by removing presentation of the drug and its associated cues. Immediately following each extinction session, rats were treated with either the HDAC inhibitor, PhB (100 mg/kg, at 1 ml/kg i.p) or a saline vehicle (0.9%; 1 ml/kg i.p). The potential for PhB to attenuate nicotine-seeking was then assessed following three reinstatement tests. **Results:** There was no evidence of a difference between the treatment groups during extinction. Administration of PhB resulted in a significant reduction in relapse to nicotine-seeking behaviour following cue reinstatment ($p = 0.01$), combined cue and drug-induced reinstatement ($p = 0.044$), and drug-induced reinstatement ($p = 0.039$). **Conclusion:** Results from this study suggest that the potential for nicotine-associated cues to induce relapse may be modulated by processes related to histone acetylation. Further studies are required to determine the precise neurobiological origin of the observed effects.

POS-MON-066

CANNABINOID MODULATION OF INPUTS TO RETINAL GANGLION CELLS

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Background: The endocannabinoid system is found throughout the brain and is implicated in many mechanisms including short-term plasticity. This is achieved by the on demand synthesis and release of cannabinoids from an excited cell, these cannabinoids then retrogradely activate presynaptic receptors and reduce neurotransmitter release. Cannabinoids and their receptors have been localised throughout the retina suggesting a possible involvement of the cannabinoid system in retinal processing. We investigated the effects of a cannabinoid agonist on the light responses in retinal ganglion cells (RGCs). **Methods:** Patch clamp recordings were obtained from RGCs to determine their effect on the strength of light responses and the spatial profile of the receptive field before and after addition of a cannabinoid agonist (WIN55212-2, 5µM). Responses were quantified in current clamp mode by measuring spike frequency and membrane potential and in voltage clamp mode by calculating inhibitory and excitatory synaptic conductances. **Results:** Cannabinoids reduced the peak response (in 21 of 23 cells) and the degree of surround inhibition (16 of 23 cells) when quantified as spikes and change in membrane potential. The conductance recordings showed that cannabinoids decreased both the excitatory (7 of 10 cells) and inhibitory (6 of 10 cells) conductance to a light stimulus. In the case of OFF alpha cells cannabinoids unmasked cross talk between ON and OFF pathways changing their light response profile to resemble that of ON-OFF cells (in 3 of 3 cells). **Conclusions:** Cannabinoids reduced the strength of light evoked responses in RGCs and modified the receptive field profile of RGCs. This effect is achieved by reducing the excitatory conductance, which occurs concomitantly with a reduction in inhibitory conductance. In one cell type, cannabinoids induced a switch in polarity. These results show that the endocannabinoid system in the retina modulates synaptic transmission, similar to its function in other areas of the brain.

POS-MON-067

D-AMPHETAMINE-SENSITIZATION IN RATS BLOCKS ACQUISITION, NOT EXPRESSION, OF RULE-GOVERNED CHOICE BEHAVIOUR

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The Stroop task is a classic tool used to assess human frontal function (McLeod, 1991; Stroop, 1935); patients with frontal deficits (e.g., schizophrenia) are impaired when responding to stimulus compounds whose elements evoke incongruent responses (Henik & Salo, 2004), suggesting impaired cognitive flexibility. By computationally modelling behavioural deficits on tasks like the Stroop, Cohen & Servan-Schreiber (1992) argued for a common underlying deficit: an inability to internally represent contextual information, something critical to successful responding on incongruent Stroop trials. **Purpose:** Featherstone, Kapur, & Fletcher (2007) argued that amphetamine-sensitization provides a good animal model of schizophrenia. We examine the effect of d-amphetamine-sensitization of rats on cognitive flexibility within the framework of the Stroop task. Specifically, we assess whether sensitization impairs performance on incongruent stimulus compounds owing to an inability to use contextual cues to guide behaviour. Also critical is the question of whether any effect of sensitization is on acquisition versus expression. **Method:** Male Long Evans rats received 7 daily injections of d-amphetamine (sensitized) or saline (control) followed by 7 injection-free days either before (n = 15 sensitized, 16 control) or after (n = 15 sensitized, 16 control) learning two biconditional discriminations, each trained within a distinct context. Individual stimulus elements from training were then presented in each context as compounds which either dictated the same response (congruent) or different responses (incongruent). Critically, incongruent trials could only be solved by utilizing previously implicit contextual cues. **Results:** Whilst animals sensitized prior to training could not solve incongruent trials, control animals and those sensitized post training could, ps <.01. **Conclusion:** Consistent with Kapur's (2003) model of schizophrenia, the effects of sensitization are on acquisition, not expression, of choice behaviour.

POS-MON-068

THE ROLE OF CONTEXT IN METHAMPHETAMINE INDUCED HABITS

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Purpose: Studies of instrumental learning suggest that early in training behaviour is goal-directed and guided by response-outcome associations. However, following extensive training, behaviour comes under the control of habits, which are governed by stimulus-response associations. Recent evidence shows that sensitization of dopaminergic systems accelerates the transition to habit dominated performance in undertrained rats. However, the influence of context on habit based performance remains to be determined. Therefore, the aim of this research was to examine the influence of a methamphetamine-paired context on goal-directed instrumental performance. **Method:** Over fourteen days, male Long Evans rats (N= 30) were administered methamphetamine (1mg/kg/d) and exposed to a distinct context (A) every other day. On alternate days rats received an equivalent volume of saline and exposed to a second context (B). Rats were then given limited training to press a lever to earn a food reward in a third context (C). Sensitivity to devaluation of the instrumental outcome was then tested in extinction, in either the methamphetamine- or saline-paired context, in drug-free states. **Results:** Animals tested in the saline-paired context displayed sensitivity to devaluation of the instrumental outcome, indexed by a significant reduction in lever pressing compared to non-devalued controls (p=.001). However, rats tested in the mAMPH-paired context showed insensitivity to devaluation of the instrumental outcome, whereby these animals continued to perform the instrumental response at comparable rates to non-devalued controls (p=.24). **Conclusion:** This finding indicates that exposure to a context previously paired with the administration of methamphetamine causes instrumental performance to come under the control of stimulus-response habits, whereas exposure to a saline-paired context allows goal-directed behaviour to be expressed.

POS-MON-069

AMPHETAMINE SENSITIZATION DISRUPTS LATENT INHIBITION AND RENEWAL OF EXTINCTION IN RATS

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Purpose: Amphetamine sensitization has been proposed as a psychopharmacological model of schizophrenia in rats. We examined the effect of amphetamine sensitization on latent inhibition and renewal. Normal latent inhibition is demonstrated by the ability of non-reinforced cue exposure to retard the subsequent development of Pavlovian conditioned responses (CRs) to that cue. Disruptions in latent inhibition reflect changes in the normal decline in processing of non-salient cues, and are observed in patients with schizophrenia. Renewal refers to the re-emergence of CRs to an extinguished cue when extinction and test are conducted in distinct contexts and reflects the contextual modulation of cues with a mixed history of reinforcement. **Methods:** Rats were given seven, daily injections of amphetamine (1mg/kg) or control vehicle, followed by a 7-day injection-free period. All then received non-reinforced exposure to one of two auditory cues (tone or noise, counterbalanced) before receiving appetitive Pavlovian conditioning to both cues. To examine renewal, two similarly treated groups received appetitive Pavlovian conditioning in context A, followed by extinction training in a different context B, followed by tests in both contexts. **Results:** Vehicle-injected rats showed normal latent inhibition and renewal. Amphetamine-sensitized rats failed to show this effect; in latent inhibition they acquired CRs to both cues at the same rate (cueXgroup: $F_{1,14}$=4.69, p<0.05); in renewal they showed equal levels of test responding in contexts A and B (contextXgroup: $F_{1,13}$=5.36, p<0.05). **Conclusion:** Amphetamine sensitization disrupts both latent inhibition and renewal in rats. This suggests that sensitization induces changes in stimulus processing, perhaps as a consequence of changes in the ability of contextual cues to modulate performance directed towards cues with an ambiguous history of reinforcement.

POS-MON-070

ACUTE METHAMPHETAMINE INCREASES CAMP-RESPONSE-BINDING-ELEMENT PROTEIN (CREB) PHOSPHORYLATION (SER133) IN THE PREFRONTAL CORTEX ONLY IN METHAMPHETAMINE SENSITIZED RATS

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Rationale: Methamphetamine abuse remains a significant problem within society, however the neural mechanisms underlying repeated use are yet to be elucidated. Previous research has suggested a role for CREB in mediating cocaine abuse, however changes to CREB signalling following methamphetamine exposure have not been documented. This study aimed to determine whether sensitization to methamphetamine altered protein-kinase A (PKA) mediated CREB phosphorylation in the Ventral Tegmental Area (VTA) or the prefrontal cortex (PFC), areas associated with changes to motivated and conditioned behaviours and integral to the addiction process. **Methods:** Male Sprague Dawley rats (N = 40) underwent repeated methamphetamine (1ml/kg intraperitoneal (i.p.) days 1 and 7; 5mg/kg i.p days 2 - 6) or saline (1ml/kg i.p.) injections for 7 days. Fourteen days later rats were challenged with an acute methamphetamine (1mg/kg i.p.) or saline (1ml/kg i.p.) injection. Thirty minutes after challenge, brains were removed and analysed by Western Blot for alterations in phosphorylated CREB (ser133). Locomotor activity was measured on days 1, 7 and 14 to measure sensitized behaviour. **Results:** A methamphetamine challenge resulted in sensitized locomotor activity in methamphetamine pre-treated animals compared to saline pre-treated rats (p = 0.01). An increase in CREB phosphorylation in the PFC was correlated with methamphetamine-induced sensitized locomotor activity (p = 0.03) but not altered in the VTA. **Conclusion:** These findings suggest that PKA mediated CREB phosphorylation following sensitization to methamphetamine is regionally specific and that phosphorylation of CREB in the PFC is important for the expression of methamphetamine sensitized behaviour.

POS-MON-071

A ROLE FOR THE MTOR PATHWAY IN THE DEVELOPMENT OF ADDICTION

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Purpose: The mammalian target of rapamycin (mTOR) is a serine threonine protein kinase implicated in dendritic synaptic protein translation including GluR1 AMPARs. Using gene pathway analysis we recently found that the mTOR-signalling pathway is the most differentially expressed in the nucleus accumbens (NAC) between addiction -vulnerable v.s. -resilient animals. In the present study we tested whether intra-NAC or systemic injections of rapamycin (RAPA), a specific mTOR inhibitor, prevented the subsequent expression of addiction-like behaviours. Methods: Rats (n=55) were trained to self-administer cocaine for two weeks. For intra-NAc infusions, RAPA was injected over 5 days (0 or 5ug/ul; n=17/group). After intra-NAC injections, animals were tested for addiction-like behaviours including ability to refrain from drug taking and motivation to self-administer drug - assessed on a progressive ratio (PR) schedule. Cue-induced reinstatement of drug-seeking (relapse-like behaviour) was also assessed after 6-8 weeks of extinction training. For systemic treatment, RAPA was injected intraperitoneally (0 or 10mg/kg/day; n = 10/group) and animals were tested for motivation to self-administer drug over three days. Results: Intra-NAC RAPA significantly attenuated addiction-like behaviours, including the ability to refrain from drug-seeking and PR breakpoints (p <0.05). Interestingly, prior treatment with RAPA also significantly attenuated relapse-like behaviour after extinction training. Systemic rapamycin treatment was found to significantly suppress PR breakpoints (p <0.05). **Conclusions:** These data suggest that NAC mTOR signalling contributes to the processes that underpin the expression of addiction-like behaviours. Given the importance of increased NAC AMPARs in these behaviours, we hypothesize that mTOR's role may involve increased GluR1 AMPAR translation.

POS-MON-072

PRAZOSIN, AN ALPHA1-ADRENOCEPTOR ANTAGONIST, PARTIALLY IMPAIRS THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO ETHANOL

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Purpose: Previous studies suggest that noradrenergic signaling may influence alcohol drinking behavior. The mechanisms underlying this effect are not clearly understood. One possible explanation could relate to noradrenergic involvement in sensitization to the stimulant effect of ethanol. In this study, we evaluated the influence of prazosin (praz) on the development and expression of behavioral sensitization to ethanol. **Methods:** The baseline activity of 78 male Swiss-Webster mice (drug-free) was measured in activity cages for 15 min. Seventy-two hours later, they were randomly allocated to receive prazosin (0.5mg/kg) or saline, 30 minutes before ethanol (2.2g/kg) or saline (i.p.) (Praz+EtOH: n=17; Praz+Sal: n=20; Sal+EtOH: n=21 and Sal+Sal: n=20). They were then tested for 15 minutes in activity cages (1st day). Mice were subsequently treated for 7 days, being tested in activity cages on the 5th, 9th and 13th days. On the 3rd, 7th and 11th days, after receiving saline or drugs they were placed in their home-cages. Days 2, 4, 6, 8, 10 and 12 were drug free. After 5 days of washout, all animals received saline and were tested in activity cages. Forty-eight hours later, all mice were challenged with ethanol (2.2g/kg). After another 48 hours, mice were challenged with the same schedule utilized during treatment. **Results:** During the development phase, the Praz+EtOH group showed lower activity than the Sal+EtOH group on days 3 and 11 (p<0.05). In the challenge phase, the Praz+EtOH pretreatment group exhibited higher levels of activity when re-challenged with ethanol alone compared to a Praz+EtOH (p<0.01) or saline (p<0.01) re-challenge. **Conclusion:** Prazosin partially impaired the development and expression of ethanol sensitization in mice.

POS-MON-073

A ROLE FOR THE mGlu5 RECEPTOR IN METHAMPHETAMINE-SEEKING?

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Purpose: Methamphetamine (METH) is a highly addictive psychostimulant. Chronic use can lead to altered glutamatergic function in both humans and rodents. The metabotropic glutamate 5 receptor (mGlu5) modulates glutamate transmission and has been implicated in drug-seeking behaviour for many drugs of abuse. We sought to assess the involvement of this receptor in METH-seeking behaviour. **Methods:** mGlu5 knockout (KO) mice and their wild type (WT) littermates were tested in behavioural paradigms relevant to drug-seeking behaviour: conditioned place preference (CPP; N = 16 WT, 22 mGlu5 KO), locomotor sensitization (N = 16 WT, 22 mGlu5 KO) and operant intravenous self-administration (N = 9 WT, 10 mGlu5 KO). **Results:** mGlu5 KO and WT mice acquired a place preference to 2mg/kg METH, but KO mice showed greater conditioned hyperactivity upon test compared to WT mice. The development of locomotor sensitization was similar between mGlu5 KO and WT mice, and the expression of sensitization upon re-challenge was comparable between genotypes. mGlu5 KO mice demonstrated no deficit in the acquisition of, or stable responding for, METH (3µg/kg/infusion) under a fixed ratio 1 schedule of reinforcement. In addition, there was no effect of genotype under a progressive ratio schedule of reinforcement. **Conclusion:** The current experiments suggest that mGlu5 signalling is not critical in the acquisition or expression of METH-induced CPP, locomotor sensitization or intravenous self-administration. These results share considerable overlap with the phenotype of mGlu5 KO mice observed in response to other psychostimulants tested in our laboratory (e.g. cocaine), and do not discount involvement of mGlu5 in acute modulation of psychostimulant actions.

POS-MON-074

THE EFFECT OF HISTONE DEACETYLASE INHIBITION ON THE EXTINCTION AND REINSTATEMENT OF METHAMPHETAMINE-INDUCED CONDITIONED PLACE PREFERENCE

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Rationale: Methamphetamine (METH) is a potent psychostimulant, the long-term use of which has severe physical and psychological consequences, including addiction and a high risk of relapse even after prolonged abstinence. Extant research indicates that relapse involves deeply entrenched memories of drug-reward, which elicit drug-seeking when triggered. Attempts to counter these memories through extinction training during addiction therapy have thus far been unsuccessful, prompting research into pharmacological extinction enhancement. Preliminary findings indicate that histone deacetylase (HDAC) inhibition is able to enhance extinction and attenuate reinstatement of cocaine and morphine seeking; as yet, however, HDAC inhibition has not been investigated in relation to METH use. The current study examined the effect of valproic acid (VPA), an HDAC inhibitor, on the extinction and reinstatement of METH-induced conditioned place preference (CPP). **Methods:** 50 male Sprague Dawley rats underwent conditioning (METH 1mg/kg i.p.) to develop a preference for a METH-paired context, followed by an extinction period to eliminate this preference. Immediately following each extinction session, 25 rats received administration of VPA (100mg/kg, i.p.) and 25 received saline (1ml/kg, i.p). Following 5 days of extinction, all rats were administered a priming injection of METH (1mg/kg, i.p.), and the extent of CPP reinstatement was assessed. **Results:** VPA treatment facilitated extinction of METH-induced CPP ($p = .019$), but did not attenuate METH-primed reinstatement of CPP. **Conclusions:** These findings suggest that HDAC inhibitors may enhance extinction training in addiction therapy, yet may not inhibit METH-induced relapse. These results likely reflect the differential strength of context- and drug-induced reinstatement, and highlight the different priming strengths of METH administration in comparison to other drugs of abuse.

POS-MON-075

CCI OF IB4+ NOCICEPTORS TRIGGERS A DECREASE IN THEIR ABILITY TO BIND IB4 AND A CONCOMITANT INCREASE IN THEIR ABILITY TO BIND AND TRANSPORT CTB

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Purpose: Primary afferent glycoconjugate expression is believed to correlate with sensory modality. The lectin Bandeiraea simplicifolia I-isolectin B4 (IB4) is used to visualize glycoconjugates uniquely expressed by small diameter nociceptors, whilst the beta subunit of Cholera Toxin (CTb) has affinity for glycoconjugates expressed by large diameter afferents. **Aim:** With a view to clarify explanations for decreases in IB4+, and increases in CTb+, axon terminals in the superficial dorsal horn after nerve injury, we combined transganglionic IB4 and CTb tracing, CCI and IB4 histochemistry in the rat to determine whether IB4+ nociceptors alter their glycoconjugate expression and become capable of expressing CTb binding glycoconjugates after injury. **Methods:** Male SD rats (n=4) were anaesthetized and both sciatics injected with IB4. After 4d rats were subjected to CCI of the left sciatic nerve and 12d later received bilateral sciatic injections of CTb. After 4d rats were perfused, lumbar DRG harvested and subjected to immuno- and lectin-histochemistry to visualize traced IB4 and CTb and binding glycoconjugates for IB4 respectively. **Results:** DRG analysis confirmed previous results that CCI reduces IB4 binding capacity of nociceptors capable of transporting IB4 prior to injury, with almost 50% of the injured IB4-traced nociceptors unable to bind IB4. Conversely, up to 70% of the injured IB4-traced nociceptors which could no longer bind IB4, exhibited de novo binding and transport of CTb. **Conclusion:** CCI decreases IB4 binding capacity and triggers concomitant *de novo* expression of the CTb binding glycoconjugate by nociceptors. Such injury induced shifts in glycoconjugate expression may underlie alterations in nociceptive processing associated with chronic pain conditions.

POS-MON-076

SENSORY AND SYMPATHETIC INNERVATION OF THE MOUSE CORNEAL EPITHELIUM

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The corneal epithelium is densely innervated, has a relatively simple epithelial structure, low metabolic demands, tissue transparency and avascularity, making it an ideal tissue to study the structure and function of nerve terminals both in vitro and in vivo. In this study, we have used whole-mount immunohistochemistry to define the morphology and neurochemistry of nerve fibres and terminals that may serve sympathetic and sensory functions in the mouse corneal epithelium (n=26). PGP9.5-like immunoreactivity (-LI) confirmed a dense innervation pattern. The sub-basal plexus consists of long nerve fibre bundles (leashes) that run in the plane of the basal epithelium, and free terminal endings with small boutons between leashes. Superficial terminals arise directly from leashes, and project up to within a few micrometers of the surface of the epithelium, where they terminate as single simple bulbar endings, horizontally ramifying fibres with simple endings, or complex endings consisting of a mix of both. TH-LI revealed a dense population of sympathetic fibres that terminate as simple endings in the sub-basal plexus and as complex ramifying endings in the superficial epithelium. CGRP-LI and SP-LI revealed a population of peptidergic sensory nerve fibres that end as thin terminals with simple bulbar endings (central cornea) or horizontally ramifying terminals with simple endings (peripheral cornea). TRPV1-LI revealed another population of sensory nerve fibres that terminate as horizontally ramifying endings in the superficial epithelium and the majority of these are non-peptidergic. We could not identify any fibres that bound IB4. Our findings suggest that subpopulations of sensory and sympathetic fibres in the mouse corneal epithelium can be defined on the basis of their morphology and neurochemistry.

POS-MON-077

THE NEUROTROPHIC FACTOR, ARTEMIN, TARGETS SPECIFIC FUNCTIONAL CLASSES OF BLADDER NOCICEPTOR NEURONS

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Purpose: Artemin is a member of the glial cell line-derived neurotrophic factor family of ligands and has important functions in development and axon regeneration. Artemin also sensitises nociceptors, potentially contributing to pain associated with injury and inflammation. The role of artemin in visceral inflammatory pain is poorly understood. The aim of our study was to determine the potential targets for artemin in the bladder nociceptive system. **Methods:** Bladder afferent neurons in lumbar and sacral dorsal root ganglia (DRG) of adult female Sprague-Dawley rats were identified with retrograde tracer. DRG and bladder tissues were immunostained for the artemin receptor (GFRα3) and markers of functionally distinct neuronal classes. All observations were made in at least 4 rats. **Results:** Many (~30%) bladder afferent neurons were GFRα3-positive, and almost all expressed transient receptor potential V1 (TRPV1) and calcitonin gene-related peptide. GFRα3-positive bladder afferent neurons more commonly expressed nitric oxide synthase in lumbar than sacral DRG (~70 vs. 30%). Many (~40%) bladder afferent neurons expressed estrogen receptor-α but only those in the lumbar DRG co-expressed GFRα3. Within the bladder, GFRα3-immunoreactive nerves were associated with detrusor muscle and blood vessels, but were relatively sparse in the suburothelium. Many of these vascular nerves coexpressed markers of noradrenergic axons. GFRα3-immunoreactivity was also found in glia. **Conclusion:** Artemin has numerous potential sites of action in the bladder, including peptidergic nociceptors, sympathetic vasoconstrictor axons and glia. Artemin potentially targets specific classes of nociceptors that differ between lumbar and spinal levels, raising the possibility that artemin modulates distinct aspects of bladder activity and pain during inflammation.

POS-MON-078

ASSOCIATIONS OF ALCOHOL CRAVING, PERSONALITY TRAITS AND CRFR1 POLYMORPHISM

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Purpose: Personality may modulate craving by mediating the selection of strategies for coping with stress. SNP rs1876831 in the corticotrophin-releasing factor receptor-1 gene (*CRFR1*) is associated with risky drinking behaviour related to negative life events. We explored personality traits that might influence alcohol craving in early-abstained alcoholic subjects, and the association of *CRFR1* rs1876831 and alcohol consumption in relation to abusive experience and personality. **Methods:** Ninety-eight alcoholic subjects (58 males, 40 females) were recruited during a 5-day in-patient detoxification program. Alcohol-use history and abusive experiences were recorded during an interview. Alcohol craving level was evaluated by Alcohol Urge Questionnaire at 7:30 a.m. around treatment day 4, followed by collection of a saliva sample for cortisol quantification. NEO PI-R Neuroticism, Extraversion and Conscientiousness traits were measured by self-report questionnaire. Genotyping was done by PCR-RFLP. **Results:** After controlling for gender, anti-depressant usage, salivary cortisol, and recent alcohol consumption, alcohol craving was found to be correlated with Neuroticism only (std B=.302; p=0.004) in a forward multiple regression model (adj r^2=.239; p<0.001). Individuals who reported ever having been abused demonstrated a significantly higher Neuroticism score regardless of gender (p=0.001). In male subjects, the interaction between abusive experience and rs187681 was significantly associated with recent alcohol consumption (p=0.037). **Conclusions:** These data suggested that neurotic personality traits is associated with increased alcohol consumption, and that rs187681 G allele may be associated with increased alcohol consumption in individuals who had experienced negative life events. A larger sample size is needed to confirm these findings.

POS-MON-079

THE RED NUCLEUS AND THE RUBROSPINAL PROJECTION IN THE MOUSE

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We studied the organization and spinal projection of the mouse red nucleus with a range of techniques (Nissl stain, immunofluorescence, retrograde tracer injections into the spinal cord, anterograde tracer injections into the red nucleus, and in situ hybridization) and counted the number of neurons in the red nucleus (3,200.9 ± 230.8). We found that the rubrospinal neurons were mainly located in the parvicellular region of the red nucleus, more lateral in the rostral part and more medial in the caudal part. Labeled neurons were least common in the rostral and caudal most parts of the red nucleus. Neurons projecting to the cervical cord were predominantly dorsomedially placed and neurons projecting to the lumbar cord were predominantly ventrolaterally placed. Immunofluorescence staining with SMI-32 antibody showed that ~60% of SMI-32-positive neurons were cervical cord-projecting neurons and 24% were lumbar cord-projecting neurons. SMI-32-positive neurons were mainly located in the caudomedial part of the red nucleus. A study of vGluT2 expression showed that the number and location of glutamatergic neurons matched with those of the rubrospinal neurons. In the anterograde tracing experiments, rubrospinal fibers travelled in the dorsal portion of the lateral funiculus, between the lateral spinal nucleus and the calretinin-positive fibers of the lateral funiculus. Rubrospinal fibers terminated in contralateral laminae 5, 6, and the dorsal part of lamina 7 at all spinal cord levels. A few fibers could be seen next to the neurons in the dorsolateral part of lamina 9 at levels of C8-T1 (hand motor neurons) and L5-L6 (foot motor neurons), which is consistent with a view that rubrospinal fibers may play a role in distal limb movement in rodents.

POS-MON-080

ELECTROPHYSIOLOGICAL PROPERTIES OF IDENTIFIED LONG DESCENDING PROPRIOSPINAL NEURONS IN MICE

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Purpose: Long descending propriospinal neurons (LDPNs) projecting from cervical to lumbar spinal cord (SC) segments are important for interlimb coordination. Recent evidence also suggests LDPNs facilitate the formation of *de novo* neural pathways that contribute to functional recovery following spinal cord injury (Bareyre et al, 2004, Nat. Neurosci., 7(3):269-77). Surprisingly, little is known about the intrinsic electrophysiological properties of LDPNs. We have therefore studied identified LDPNs in the mouse SC using targeted, whole-cell patch clamp electrophysiology. **Methods:** Mice (C57Bl6, 22-27 days) were anaesthetized (1-3% isoflurane, 0.5 L/min) and injected with a fluorescent retrograde tracer (DiI) in the lumbar SC (L1/L2) to identify LDPNs projecting to this region. After 48 hours, mice were overdosed with Ketamine (100 mg/kg, i.p.) and transverse slices (300 µm thick) of rostral SC were obtained. Targeted, whole-cell patch clamp recordings (23°C, KCH_3SO_4-based internal) were then made from fluorescent LDPNs with cell bodies in cervical and upper thoracic SC segments (C6-T2). **Results:** Recordings were obtained from 12 identified LDPNs and 8 control (non-fluorescent) neurons (RMP -36.6 ± 2.7mV vs. -45.4 ± 1.4mV, R_{in} 602.2 ± 244.5MΩ vs. 515 ± 95.1MΩ, M_{cap} 27.3 ± 2.9pF vs. 15.9 ± 1.3pF). Compared to control recordings, LDPNs were more likely to discharge action potentials (APs) spontaneously at rest (75% vs. 38%) and exhibit a tonic AP discharge pattern (80% vs. 38%) in response to depolarizing current steps (1s duration, 20pA increments). Finally, 88% of LDPNs (vs. 66% control) exhibit an I_h-like inward current. **Conclusions:** Spontaneous AP discharge, a tonic firing pattern, and I_h-like inward currents are characteristic of LDPNs. These properties are consistent with data from other neuronal populations known to participate in rhythmic motor behaviours.

POS-MON-081

THE RAT WHISKER M1 AREA CAN EVOKE WHOLE MOVEMENT PATTERNS WITH SUCCESSIVE TRAINS OF STIMULATION

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Background: The rat's facial whiskers, its major sensor to detect, recognise and discriminate objects and navigate complex spaces, is controlled from an M1 area in which intracortical microstimulation (ICMS) is reported to evoke only whisker movement under light anaesthesia (Tandon et al., 2008) or in conscious animals (e.g., Haiss and Schwarz, 2005; Brecht et al., 2004). This area is divided into a "retraction-face" region where ICMS, in awake animals, causes a twitch pulling all whiskers backward, and a Rhythmic Whisking region which evokes, in conscious animals, rhythmic whisker movements mimicking voluntary whisking, and in anaesthetized animals, large sustained whisker protractions (e.g., Haiss and Schwarz, 2005; Cramer and Keller, 2006). The specificity of whisker-only activation from this M1 area mimics early models of specificity of muscle action evoked from primate M1 but contrasts against recent reports (Graziano and Aflalo, 2007) that ICMS of behaviourally-relevant duration in awake primate M1 causes complex multi-muscle/limb movements like those within the monkey's normal behaviour. **Results**: We examined this concept in halothane-anaesthetized rats (n=8) and report that behaviourally-relevant ICMS of the M1 whisker area can, under ideal conditions of depth of anaesthesia and void of over-stimulation effects, produce movement programs. Stimulation with 10-20 x 1-sec long trains (bipolar 150 µsec pulses; 50 Hz; 20-100 µA) could produce a gradual build-up from small rhythmic whisking of 1-3 whiskers through to large whisking of most or all whiskers, often bilaterally, and then often resolving into a sustained large protraction of all whiskers. This repertoire models the rat's exploration of an object. The movement program could even extend to rhythmic movement of fore- and hind-limbs in a walking pattern. **Conclusion**: Short successive ICMS of M1 can evoke an entire motor repertoire in rat, even under anaesthesia, as reported with ICMS of behaviourally-relevant duration in awake primates.

POS-MON-082

SPONTANEOUS CLUSTER ACTIVITY IN THE INFERIOR OLIVARY NUCLEUS IN BRAINSTEM SLICES FROM POSTNATAL MICE

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Purpose: A distinctive property of the cerebellar system is olivocerebellar modules, where synchronized electrical activity in neurons in the inferior olivary nucleus (IO) evokes organized activity in the cerebellar cortex. However, the exact function of these modules, and how they are developed, is still largely unknown. **Methods**: We used calcium imaging in brainstem slices of from of P0.5-P15.5 mice (n=54) to reveal spontaneous spatiotemporal activity patterns in the IO. **Results**:The IO spontaneously generates clusters of neurons with synchronous Ca^{2+} transients. Neurons in the principal olive (PO), and the vestibular-related dorsomedial cell column (dmcc), showed an age-dependent increase in spontaneous calcium transients. The spatiotemporal activity pattern was occasionally organized in clusters of co-active neighboring neurons, with regular (16/min) and irregular (2-3/min) repeating cluster activity in the dmcc and PO, respectively. IO clusters had a diameter of 100-170 µm, lasted ~1 s, and increased in occurrence from P5.5-P12.5, followed by a sharp drop to near zero at P15.5. IO clusters were overlapping, and comprised of near identical neurons at some times, but a varied subset of neurons at other times. Some neurons had hub-like properties, being co-active with many other neighbors, and some were co-active with separate clusters at different times. The coherence between calcium transients in IO neurons decreased with Euclidean distance between the cells reaching low values at 100-200 µm distances. Intracellular recordings from IO neurons during cluster formation revealed the presence of spikelet-like potentials, suggesting that electrical coupling between neighboring IO neurons may serve as a synchronizing mechanism. **Conclusion**: The IO shows spontaneous cluster activity under in vitro conditions, coinciding with a critical postnatal period in olivocerebellar development.

POS-MON-083

THE INFERIOR OLIVE OF THE C57BL/6J MOUSE - CYTOLOGY, CELL NUMBERS, AND THE OLIVE-PURKINJE CELL RATIO

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PURPOSE: The olivocerebellar system of the mouse is of interest to those analysing the effect of knockout of relevant genes on inferior olive and Purkinje cell development. This study provides a comprehensive description of the cytoarchitecture of the mouse inferior olive combined with an analysis of olive and Purkinje cell numbers. METHODS: We studied Nissl, AChE, and protein antibody stained sections from our own laboratory (N=5) and sections of ISH preparations showing gene expression from the Allen Brain Institute website using the AGEA tool (N=10). RESULTS: The subnuclei of the inferior olive of the C57BL mouse are very similar to those in other placental mammals, except that an arcuate nucleus is present. Some subnuclei can be distinguished on the basis of histochemical staining (AChE staining (dorsal, medial), calretinin (lateral part of principal), and tryosine hydroxylase (medial). In addition, the cells of the arcuate and ventral tier of the principal nucleus (IOPR) were smaller than those of other subnuclei.The average number of cells in one inferior olive is 8,828 The average number of Purkinje cells in a hemicerebellum is 62,705 so that the average inferior olive-Purkinje ratio (IO:Pk) is 1:7.1, which is similar to that found in the cat and the rat. A number of genes are selectively expressed in the inferior olive (CART in ventral tier of IOPr and the cap of Kooy; galanin in the caldal medial subnuclei, arcuate nucleus, and the ventral tier of IOPR) but many other genes (e.g. Pou4f1 and Calb) are expressed in all parts of the inferior olive. CONCLUSION: We have documented the organisation of the mouse inferior olive and the characteristics of individual subnuclei. Our counts of inferior olive and Purkinje cell numbers reveal that the IO:Pk ratio in the C57/BL mouse is typical of small mammals.

POS-MON-084

FUNCTIONAL CHARACTERISATION OF LAYER 5 PROJECTION NEURONS IN THE MOUSE MOTOR CORTEX

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Purpose: In the primary motor cortex (M1), the circuit output is communicated by layer 5 projection neurons (L5 PNs) to pyramidal (corticospinal) and extrapyramidal targets (i.e. basal ganglia, contralateral cortex). These complex outputs both drive and refine movement. A systematic description of M1 L5 PNs types and their targets is therefore essential. Here, we determine the electrophysiological properties of L5 PNs in M1 using a genetically encoded marker for cortical PNs combined with retrograde labelling from long-range projection targets. **Methods:** Corticospinal, corticostriatal and corticocortical PNs were labelled by stereotaxic injection of Alexa647-tagged cholera toxin or microRuby into the lumbar spinal cord, contralateral striatum or contralateral M1, respectively, at P18-21. After 3-12 days retrograde labelled PNs were visually targeted for whole-cell recording in acute slices. **Results:** Electrophysiological features (input resistance, action potential decay, afterhyperpolarisation following high frequency firing, spike frequency adaptation and hyperpolarisation-induced inward rectification) of corticostriatal (n=9) and corticocortical (n=3) PNs were similar (all $p>0.05$, one-way ANOVA, Tukey's post-hoc analysis) except for action potential amplitude and rate of rise that were smaller in corticostriatal PNs (both $p<0.001$). In contrast corticospinal PNs (n=9) exhibited a lower input resistance, lower spike frequency adaptation, a larger amplitude afterhyperpolarisation and hyperpolarisation-induced inward rectification (all $p<0.05$). Action potentials in corticospinal PNs were fastest to decay, largest in amplitude, exhibited a lower threshold and were followed by larger depolarising afterpotentials (all $p<0.05$). **Conclusion:** We identified an electrophysiological phenotype of corticospinal PNs distinct from PNs targeting commissural motor structures. This implies that unique information output is transmitted to downstream elements in the pyramidal and extrapyramidal motor pathways.

POS-MON-085

ALTERED LEVELS OF DENDRITIC ACTIN-BINDING PROTEINS IN ALZHEIMER DISEASE

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Alzheimer disease (AD) is a progressive neurodegenerative disorder characterised clinically by impaired cognition and memory, and pathologically by β-amyloid plaques, neurofibrillary tangles (NFTs), and regionally-selective loss of neurones, dendrites and synapses. The latter correlate best with the cognitive decline measured ante mortem. Synaptic dysfunction may be a major substrate of clinical symptoms. We quantified the levels of synaptophysin, a presynaptic vesicle protein; actin-depolymerising factor (ADF), an actin-binding protein concentrated in dendrites where it regulates the actin cytoskeleton with its partner, cofilin by quantitative western blotting in preparations of frozen human autopsy brain tissue using recombinant purified proteins as standards. We investigated areas susceptible to AD damage, hippocampus and inferior temporal cortex; and the relatively spared occipital cortex. Synaptophysin levels were lower in AD cases (n = 8) than in controls (n = 9) across all areas, albeit not significantly. ADF and cofilin displayed opposing trends; ADF levels were higher in AD hippocampus (n = 6) and occipital cortex than the same areas in controls (n = 6), but lower in inferior temporal cortex of AD cases. Cofilin levels were lower in the hippocampus and occipital cortex of AD cases but higher in the inferior temporal cortex compared with controls. The hippocampus had the highest levels of ADF and cofilin compared with the inferior temporal and occipital cortices. The altered levels of cofilin and ADF in different areas of the AD brain may be due to inclusions, or dendritic pruning, or dystrophic neurites. Further investigation with the progression of other markers of AD pathology is warranted. ADF and cofilin may play a role in dendritic regulation in AD.

POS-MON-086

SYNTHETIC METALLOTHIONEIN-MIMETIC PEPTIDES PROTECT AGAINST β-AMYLOID INDUCED NEUROTOXICITY

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Purpose: Alzheimer's disease is characterised by the extracellular accumulation of plaques, comprised predominantly of fragments of the β-amyloid peptide (Aβ). The Aβ peptide has also been observed to assemble into a variety of soluble forms, some of which exhibit potent neurotoxicity. We have previously found the metallothionein 2 (MT2) is protective against certain forms of Aβ, including copper-bound Aβ (Cu-Aβ) that is a known neurotoxic form of Aβ. We wished to test if emtinB, a synthetic peptide based on the MT2 peptide sequence, is also neuroprotective against Cu-Aβ. Furthermore, we hypothesised that metallothioneins and emtins might act via receptors of the Lipoprotein Receptor Related Protein class (LRPs). **Methods:** We established primary rodent hippocampal neurons in culture, and used an Alamar Blue assay to analyse the effect of copper-bound Aβ on neuronal viability in the presence and absence of emtinB. Alamar assay was also used to determine the effect of LRP inhibition on emtinB action. **Results:** We found that Cu-Aβ reduced neuronal viability to 53% (+/- 1.0) of hippocampal neurons. Administration of the MT2 analogue emtinB significantly diminished neurotoxicity of Cu-Aβ, increasing neuronal viability to 71% (+/- 2.0) and 82% (+/- 2.3) of control at doses of 10uM and 25uM respectively. Furthermore, in a direct comparison of 10uM emtinB (85% +/- 8.4) and 10uM MT (95%+/- 1.4), emtinB demonstrated an efficacy equivalent to MT2. Inhibiting the LRP family of receptors resulted in a subsequent loss of emtinB neuroprotective capacity (LRP inhibited = 76% +/-3.3, LRP active = 94% +/- 2.3). **Conclusion:** We report that emtinB can protect against the neurotoxic activity of Cu-Aβ. This neuroprotection appears to be mediated through the LRP family of receptors which, interestingly, are the putative receptors for MT2. We believe that emtinB may have therapeutic potential for some aspects of Alzheimer's Disease.

POS-MON-087

DENERVATION OF THE OLFACTORY BULB LEADS TO DECREASED AMYLOID PLAQUE LOAD IN APP/PS1 MICE

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Purpose: This project aims to determine how functional and synaptic input influence the susceptibility of the brain to beta amyloid misprocessing that ultimately leads to Abeta plaque formation. The olfactory system is particularly advantageous because olfactory input into the CNS can be manipulated by experimental ablation, thus giving us the opportunity to study in vivo the influence of neuronal activity on Abeta plaque formation in the cortex and hippocampus. **Methods:** Olfactory input was disrupted by damaging the olfactory epithelium ipsilaterally with TritonX solution in the APP/PS1 mouse model of Alzheimer's disease. Mice were subjected to nasal washes 8 times, at 3-week intervals either with 1%TritonX solution (n= 4) or phosphate buffered saline (PBS, n=4). Three days after the final treatment, mice were perfused and the brains including olfactory bulbs (OB) were processed for cryostat sectioning. Amyloid plaques were identified by Thioflavine S staining, and amyloid precursor protein (APP) was demonstrated by immunofluorescence. **Results:** Denervation of the OB resulted in a significant decrease in Abeta plaque load in the OB (0.378+ 0.078% [TritonX] vs 1.292+ 0.142% [PBS]; $p<0.01$) and cerebral cortex (2.456+ 0.195% [TritonX] vs 6.423+ 0.694% [PBS]; $p<0.01$). A decreasing trend in plaque load was observed in the hippocampus. Immunoreactivity for APP was demonstrated in mitral cells which are normally postsynaptic to olfactory neurons. Although there was no statistically significant difference in the number of mitral cells expressing APP between the two groups, there was increased immunolabelling for APP within mitral cells in the denervated OB. **Conclusion:** Denervation of the OB in APP/PS1 mice may affect APP processing which leads to a decrease in amyloid plaque load in the CNS.

POS-MON-088

EFFECTS OF GLYCOSAMINOGLYCANS ON THE LEVEL OF β- AND α-SECRETASE AND APP PROCESSING IN TG2576 PRIMARY CORTICAL CELLS

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Glycosaminoglycans (GAGs) have been proposed as therapeutic agents for the treatment of Alzheimers disease (AD). Our previous studies have shown that GAGs can bind to and stimulate the β-site APP cleaving enzyme-1 (BACE1), which catalyses the first step in the production of the β-amyloid protein (Aβ) of Alzheimers disease. Enoxaparin, a low molecular weight form of the GAG heparin, was reported to lower Aβ plaque deposition and improve the cognition in β-amyloid precursor protein (APP) transgenic mice. In the present study, we examined whether heparin and enoxaparin can influence the APP processing and inhibit Aβ production in cortical cell cultures. Heparin and enoxaparin were incubated with primary cortical cells from APP (SW) Tg2576 mice which express human APP, and the level of APP and proteolytic products of APP (sAPPα, C99, C83 and Aβ) were measured by western blotting. Treatment of the cells with heparin or enoxaparin had no significant effect on the level of total APP. However, both GAGs decreased the level of C99 and C83, and inhibited sAPPα and Aβ secretion. Heparin also decreased the level of β-secretase (BACE1) and α-secretase (ADAM10). In contrast, heparin had no effect on the level of ADAM17. We conclude that heparin and enoxaparin lower Aβ secretion from cortical cells by decreasing BACE1 and thereby inhibiting β-secretase processing of APP. This effect is not specific for amyloidogenic processing of APP, as heparin and enoxaparin also decrease the α-secretase ADAM-10 and inhibit α-secretase processing of APP.

POS-MON-089

AMYLOID PRECURSOR PROTEIN-MEDIATED NEUROPROTECTION IN THE AGED RETINA

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Purpose: Increasing age is a major risk factor for neuronal loss in Alzheimer's disease and retinal ganglion cell (RGC) loss in glaucoma. Oxidative stress associated with aging is known to increase amyloidogenic processing (beta cleavage) of the amyloid precursor protein (APP) resulting in elevated levels of the toxic amyloid beta, which has recently been found to be increased in the inner retina of rodent glaucoma models. Increased beta cleavage processing corresponds to decreased production of the neurotrophic alpha-cleavage APP product, sAPPα, and we hypothesize that loss of this trophic factor may render aged RGCs more susceptible to injury. **Methods:** Cell culture experiments were used to test whether sAPP could protect human neuronal (SHSY) cells against the mitochondrial complex I toxin rotenone, which has been shown to preferentially kill RGCs in rodents. Donor human eyes (n=18) were analysed for APP cleavage products using western immunoblotting of retina and vitreous fluid. Rodent eyes were also examined for APP expression using immunohistochemistry. **Results:** sAPPα was neuroprotective against rotenone insult in SHSY cells, ($p<0.001$) and this protection was linked to activation of the protective Akt kinase ($p<0.05$). In human retina, total APP was decreased with age and beta cleavage was increased ($p<0.05$). Rodent retina showed APP expression was limited to the RGC layer while both rodent and human vitreous fluid contained high levels of sAPPα. **Conclusion:** Soluble APPα in the vitreous may provide a neuroprotective function exerted at the inner retina.

POS-MON-090

SUPPRESSION OF CCL2 IN MULLER CELLS USING TARGETED SIRNA REDUCES PHOTORECEPTOR DEATH FOLLOWING LIGHT-INDUCED RETINAL DEGENERATION

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AIM: Recruitment of inflammatory cells into the injured retina is thought to exacerbate photoreceptor death in retinal degeneration. Monocyte recruitment is dependent on expression of chemo-attractants such as Ccl2. We investigated whether targeted knockdown of Ccl2 expression in Müller cells using RNA interference (RNAi) suppresses monocyte/microglia infiltration and reduces cell death, in a light-mediated model of retinal degeneration. **METHODS:** Adult SD rats were exposed to 1000lx of light for a period of 24hrs, after which the animals were euthanized. Immediately prior to light damage 1µg of either Ccl2 siRNA, or scrambled siRNA, complexed with Invivofectamine agent, was injected intravitreally. Retinas were harvested and evaluated for the effects of bright-light exposure. Ccl2 expression was assessed by qPCR (n=8), immunohistochemistry (n=4), and in situ hybridization (n=4). Monocytes/microglia were counted on retinal cryo-sections immunolabeled with ED1 and IBA1 (n=4), and photoreceptor apoptosis was assessed using TUNEL (n=4). **RESULTS:** Intravitreal injection of Ccl2 siRNA significantly reduced the expression of Ccl2 following light-damage to 29% compared to controls ($P<0.05$), as indicated by qPCR, while scrambled siRNA injections showed no change. In situ hybridisation and immunohistochemistry for Ccl2 on retinal cryosections showed a substantial decrease in expression in Ccl2 siRNA injected retinas ($P<0.05$). Cell counts showed significantly fewer ED1- and IBA1-positive cells in the retinal vasculature and ONL of Ccl2 siRNA-injected retinas ($P<0.05$), compared to scrambled siRNA and controls. There was significantly less photoreceptor apoptosis in Ccl2 siRNA-injected retinas compared to scrambled siRNA and controls ($P<0.05$). **CONCLUSION:** Our data indicate that Ccl2 expression by Müller cells promotes the infiltration of monocytes/microglia, thereby contributing to the neuroinflammatory response and photoreceptor death following retinal injury.

POS-MON-091

INCREASED MOTILITY IN NEUROSPHERE-DERIVED CELLS FROM SCHIZOPHRENIA PATIENTS IS MEDIATED VIA FOCAL ADHESION KINASE SIGNALLING

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Purpose: Schizophrenia is a strongly heritable multifactorial mental disorder whose aetiology is not understood. Lack of identified causative genes and the limits of animal models have impeded the understanding of this disease from the biological perspective. There is a consensus, based on epidemiology and post-mortem human brains that schizophrenia is a neurodevelopmental disorder, but cellular and molecular mechanisms for that hypothesis are lacking. We developed a new model to address this using olfactory neurosphere-derived (hONS) cells, a novel method which allows comparative analyses between age- and sex-matched schizophrenic patients and healthy subjects. Gene expression profiling identified focal adhesion kinase (FAK) signalling pathway as being significantly dysregulated in schizophrenia in hONS cells. **Methodology:** This aim of the present study was to determine whether dysregulated FAK signalling would have consequences on cell motility. hONS cells were generated from olfactory mucosal biopsies isolated from healthy control and patients with schizophrenia (n=9 per group). hONS cell motility was quantified in microfluidic migration devices over a 20 h time period ± FAK Inhibitor 14 (3 µM). Total and phosphorylated FAK protein expression was quantified using Western blot ± FAK Inhibitor 14 (3 µM; n=9 per group and 1-4 µM; n=3 per group). **Results:** Patient-derived hONS cells exhibited a faster migration rate in these microfluidic devices. FAK Inhibitor 14 suppressed patient hONS cell migration without affecting the migration rate of control hONS cells. FAK Inhibitor 14 treatment reduced phosphorylated FAK expression in both patient and control hONS cells. **Conclusion:** These results demonstrate that hONS cells from patients with schizophrenia have altered motility mediated by FAK signalling. This is the first report of altered motility in neural cells from patients with schizophrenia and demonstrates the utility of this model in identifying signalling pathways that may lead to altered neurodevelopment in this disorder.

POS-MON-092

THE EFFECT OF A PERINATAL IMMUNE CHALLENGE IN NRG1 MUTANT MICE ON NMDA AND GABA RECEPTOR DENSITY IN SCHIZOPHRENIA-RELEVANT BRAIN AREAS

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Schizophrenia is a devastating brain disorder. Two of the major factors shown to increase the risk to develop schizophrenia are genetic predispositions and early-life infections. Whereas each factor on its own does not sufficiently explain schizophrenia, gene-immune interactions are increasingly suggested to facilitate disease development. **Purpose** In this study, we investigated the effect of a mutation of the major schizophrenia candidate gene Neuregulin-1 (Nrg1) in interaction with an early-life immune challenge. **Methods** For this, we treated Nrg1 mutant mice (n=12) and wild type controls (n=12) perinatally with PolyIC, mimicking a viral infection in early brain development, compared to saline controls. In early adulthood, we studied the density of NMDA and $GABA_A$ receptors in several brain areas relevant for schizophrenia, including the prefrontal cortex, hippocampus and striatum. **Results** $GABA_A$ receptor density in the hippocampus was significantly affected by PolyIC treatment. $GABA_A$ receptor density was tendentially increased due to PolyIC treatment in wild types ($p=0.06$) and significantly increased in Nrg1 mutant mice ($p<0.01$). In the PFC, PolyIC treatment of Nrg1 mutant mice showed a strong tendency to increase $GABA_A$ receptors compared to controls ($p=0.07$). We did not find any differences in NMDA receptor density due to genotype or treatment in the studied brain areas. **Conclusion** These results indicate that an early-life immune challenge when interacting with a genetic predisposition can alter neurotransmitter receptors as relevant for the schizophrenia pathology. Further research will focus on associated neuroimmune factors, behavioural effects and examine the potential of antipsychotic drugs to prevent these changes.

POS-MON-093

ALTERATIONS IN GENE EXPRESSION OF NEUREGULIN-1 AND INTERLEUKIN-6 SIGNALLING MOLECULES IN IMMUNE CHALLENGED NEUREGULIN-1 MUTANT MICE IN SCHIZOPHRENIA-RELEVANT BRAIN AREAS

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Schizophrenia is a devastating brain disorder with unknown aetiology. Gene-immune interactions are increasingly suggested to facilitate disease development. Neuregulin-1 (Nrg1) is a major candidate gene for schizophrenia, and the cytokine Interleukin-6 (IL-6) was found altered in schizophrenia patients. Increased IL-6 levels were recently reported in both human and murine Neuregulin (Nrg1) mutants. **Purpose:** To study this gene-immune interaction, we investigated gene expression along the Nrg1 and IL-6 signalling pathways in schizophrenia-relevant brain regions in the Neuregulin-1 heterozygous mutant (Nrg1 HET) mouse model. **Methods:** We treated adult Nrg1 mutant mice (n=6) and wild type littermates (n=6) with B16F0 cells, established to induce a chronic immune challenge and increase IL-6 release. After 9 days, brain tissue was removed and analysed for mRNA levels using a Nrg1 and IL-6 signalling RTqPCR array. **Results:** In controls, we found a significant increase in SOCS3 mRNA (p=0.013) in the prefrontal cortex of Nrg1 HETs. Following the immune challenge, we found IL-6Rα (p=0.034), ErbB2 (p=0.001) and Pten (p=0.030) mRNA levels increased in the prefrontal cortex, and Jak1 (p=0.025) mRNA levels decreased in the hippocampus of Nrg1 HETs. **Conclusion:** Together these data indicate that an interaction of a Nrg1 mutation and an immune challenge can affect Nrg1 and IL-6 signalling to potentially change the neuronal activity in schizophrenia-relevant brain areas.

POS-MON-094

CHARACTERIZATION OF A NOVEL KV11.1 POTASSIUM CHANNEL ISOFORM UPREGULATED IN SCHIZOPHRENIA PATIENTS

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Purpose Recently, Weinberger and colleagues found an association between single nucleotide polymorphisms (SNPs) in the second intron of *KCNH2*, which encodes the Kv11.1 K^+ channel, and schizophrenia (Huffaker et al., NatMed,2009,15(5):509-18.). The minor allele of these SNPs result in increased expression of an alternatively spliced Kv11.1 isoform (known as Kv11.1-3.1) in the prefrontal cortex and hippocampus. Intriguingly, the vast majority of antipsychotics, irrespective of their primary target, also inhibit Kv11.1 K^+ channels. This raises the possibility that, in patients who have increased levels of Kv11.1-3.1, treatment with antipsychotics could exacerbate, or ameliorate, symptoms of the disease. Accordingly, the aim of this study was to characterize the gating properties of the novel Kv11.1-3.1 isoform and investigate its sensitivity to antipsychotic drugs. **Methods:** CHO cells were transfected with Kv11.1 K^+ channels and currents recorded using the whole-cell voltage clamp technique. **Results & Conclusions:** Kv11.1-3.1 displays the same voltage dependence of channel activation as the 1A isoform, but the voltage dependence of channel inactivation is shifted 23 mV more positive than for the 1A isoform. The rates of recovery from channel inactivation and rates of channel deactivation are also significantly faster for the 3.1 isoform compared to the 1A isoform. Overall these properties lead to less accumulation of outward current during repetitive stimulation, i.e. a loss of function compared to the 1A isoform. Kv11.1-3.1 channels are also less sensitive to block by Haloperidol compared to the 1A isoform. This could have implications for how patients with increased expression of the 3.1 isoform respond to antipsychotic drugs.

POS-MON-095

INFLAMMATORY GENE EXPRESSION AND MICROGLIAL DENSITY ARE UPREGULATED IN THE DLPFC OF SCHIZOPHRENIA

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Purpose: Schizophrenia is a psychiatric disorder characterized by hallucinations and delusions that has been linked to abnormal inflammatory responses. **Methods:** Next-generation sequencing of an Australian cohort, 20 males with schizophrenia and 20 matched controls, showed inflammatory pathway genes were up-regulated in schizophrenia DLPFC. SOLiD RNA-Seq libraries were prepared and sequenced resulting in 135 million 50 base pair reads per individual. Mapping, differential expression, and gene set enrichment analysis was performed using X-Mate, Galaxy and edgeR respectively, with genes showing <0.05 FDR exported to Ingenuity for pathway analysis. Up-regulation of identified inflammatory genes was confirmed through qPCR, while western blotting and immunohistochemistry were used for microglial protein analysis. **Results:** 115 genes were inputted into Ingenuity suggesting an inflammatory network. Specific inflammatory transcripts such as SERIPINA3, IL6, and IL8 were confirmed to be significantly upregulated (p<0.05). Significant increases in protein levels of the MHC-II complex, a microglia marker, were found in homogenate and in the white matter reflecting an increase in microglia density (p<0.05). **Conclusion:** We identified a widespread up-regulation of immune related genes that provides evidence of an increased inflammatory process in ~32% of patients with schizophrenia. Although it is unclear if these changes are a core component of the disease, a compensatory response or a side effect of medications they suggest promising therapeutic targets.

POS-MON-096

DIAGNOSTIC-SPECIFIC REDUCTIONS IN NMDA AND MGLU5 GLUTAMATERGIC RECEPTORS IN SCHIZOPHRENIA

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There is strong evidence supporting the involvement of the glutamatergic system, particularly NMDA receptors, in the pathophysiology of schizophrenia. However, due to the heterogeneity of the disorder, the role of the glutamatergic system in schizophrenia and its specific subclasses remains unclear. **Purpose:** This study aimed to examine ionotropic and metabotropic glutamate receptors in a large cohort of schizophrenia subjects, taking into consideration subclass and comorbidity. **Methods:** Using *in situ* quantitative receptor autoradiography, the binding densities of NMDA receptors, NR2A- and NR2B-containing NMDA receptors, and mGlu2/3 and mGlu5 receptors were examined in the dorsolateral prefrontal cortex (DLPFC) of schizophrenia (n=37) and control (n=37) subjects. **Results:** NMDA receptor binding density was reduced in the DLPFC of subjects specifically diagnosed with schizoaffective disorder (-15%, p=0.018) and accordingly also reduced in subjects with comborbid major depression (-14%, p=0.006). Similarly, mGlu5 binding density showed a 31% reduction in subjects with comorbid major depression compared to controls (p=0.034), with a trend towards a reduction in schizoaffective patients (-20%, p=0.079). A positive correlation was observed between NMDA and mGlu5 binding, which was especially significant in schizophrenia subjects (r=0.520, p=0.001). Binding of NR2B-containging NMDA receptors and mGlu2/3 receptors remained unchanged. **Conclusion:** Our findings indicate a reduction in both NMDA and mGlu5 receptors in the DLPFC of schizophrenia/schizoaffective subjects with comorbid major depression. These results provide support for the NMDA/mGlu5 receptor complex as a pharmaceutical target for the treatment of schizophrenia, at least in specific cases of schizoaffective disorder and major depression comorbidity. The findings from this study emphasize the heterogeneity of schizophrenia pathology, which must be considered in future studies if we are to unravel the biological complexity of schizophrenia.

POS-MON-097

METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 IS REDUCED IN SCHIZOPHRENIA SUBJECTS WITH COMORBID MAJOR DEPRESSION AND UNCHANGED FOLLOWING ANTIPSYCHOTIC DRUG TREATMENT

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Purpose: To examine mGluR5 expression in the dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects compared to control subjects. Additionally, to investigate whether antipsychotic drug (APD) treatment causes adaptive changes in mGluR5. ***Methods:*** Using post-mortem human brain tissue, mGluR5 binding and protein density were measured in the DLPFC of schizophrenia subjects (n=37) and matched control subjects (n=37) using autoradiography and western blot methods respectively. Subsequently, rats treated with haloperidol, olanzapine or vehicle, for short-(8 days), medium-(15 days) or long-term (35 days) durations (n=6/group) were tested for alterations in mGluR5 binding levels in the PFC by receptor autoradiography. ***Results:*** Subjects comorbid with major depression (the majority of which suffered schizoaffective disorder) displayed a 31% decrease in mGluR5 binding density (p=0.014). mGluR5 protein level was unaltered. mGluR5 did not correlate with pre-mortem APD or antidepressant treatment. Finally, mGluR5 binding was unaltered in the PFC of APD treated rats. ***Conclusion:*** This study found reductions in mGluR5 binding in the DLPFC of schizophrenia subjects, specifically in those with comorbid major depression. However, as protein levels were unaltered, this suggests a possible alteration in receptor function rather than receptor numbers. This study highlights that alterations in mGluR5 receptors vary with symptomatology, which is paramount information to include in future studies. In addition, this study points towards a role of mGluR5 as a novel pharmaceutical target for the treatment of schizophrenia, at least in cases of specific symptoms associated with subdiagnosis and comorbidity, especially as it was found in this study that current therapeutics do not influence mGluR5.

POS-MON-098

DIFFERENTIAL GENE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM A LARGE COHORT OF PARTICIPANTS WITH SCHIZOPHRENIA

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Purpose: Peripheral blood mononuclear cells (PBMCs) can be easily obtained from living patients, representing an accessible non-neuronal tissue alternative to brain tissue for the possible development of a biological means for diagnosis or subtype classification of schizophrenia. **Methods:** PBMCs were isolated from whole blood from 115 patients with schizophrenia or schizoaffective disorder and 80 non-psychiatric controls from the Australian Schizophrenia Research Bank and mRNA expression measured using Illumina HT_12 microarrays. Differential expression was validated by qPCR and Ingenuity Pathways Analysis used to infer functional changes. **Results:** Differential expression of 167 genes was detected in PBMCs from participants with schizophrenia relative to controls (106 downregulated, 61 upregulated). Five genes were validated as being significantly altered ($p < 0.05$) with another two showing a strong non-significant trend. Functional analyses suggests the differentially expressed genes are significantly enriched in pathways for immune and infection-related processes. Several of the altered genes fall within previously reported schizophrenia linkage sites. **Conclusions:** The differential gene expression supports a role for immune system dysfunction in schizophrenia consistent with a previous analysis of miRNA expression in the same cohort of participants. The functional implications of this immune-related expression signature in schizophrenia are yet to be determined. Ongoing studies are attempting to validate the utility of this approach in developing a molecular signature for identifying subtypes of schizophrenia.

POS-MON-099

MINOCYCLINE TREATMENT ATTENUATES AXONAL PATHOLOGY AND MOTOR DEFICITS IN A MOUSE MODEL OF INDUCIBLE OLIGODENDROCYTE APOPTOSIS

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Purpose: Recent studies of newly forming multiple sclerosis (MS) lesions have demonstrated that oligodendrocyte (OL) apoptosis and microglial activation could play crucial roles in pathogenesis. To model OL apoptosis, we developed the MBP-DTR^{100A} transgenic mouse model in which expression of the diphtheria toxin receptor (DTR) is regulated by the proximal promoter of mouse myelin basic protein (MBP). Administration of diphtheria toxin (DT) to MBP-DTR^{100A} mice induces OL apoptosis, microglial cell activation and axonal damage independent of demyelination and lymphocyte infiltration, reflecting early MS lesion pathology. The present study aimed to investigate whether suppression of microglial cell activation attenuates motor deficits and axonal pathology in this model. **Methods:** Experiments comprised four experimental groups consisting of transgenic or wild-type littermates administered a single DT injection (10µg/kg, i.p.) in conjunction with daily injections of either the anti-inflammatory drug minocycline (50mg/kg/day, i.p.) or PBS (vehicle). Changes in body weight, motor function, OL and microglial cell density and the integrity of nodes of Ranvier were assessed. **Results:** Minocycline treatment attenuated the development of early motor deficits in transgenic mice (n=8, $p<0.05$) and delayed the progression of clinical symptoms relative to vehicle-treated controls. Increased microglial cell density observed pre-clinically following DT administration was attenuated by minocycline treatment. At clinical peak, extensive OL loss was accompanied by a decline in microglial cell density in vehicle-treated transgenic mice, suggesting an autoregulatory response within the microglial population. At the axonal level, lengthening of ankyrin G-immunolabelled nodes was abolished in minocycline-treated transgenic mice. **Conclusion:** Microglial cell activation in response to DT-mediated OL apoptosis likely contributes to axonal damage. Future studies will identify the molecular mechanisms underlying microglial-induced axonal pathology.

POS-MON-100

LOCALISATION OF PTHR555CRMP-2 IN DEGENERATIVE SOMATA AND AXONS IN CHRONIC-ACTIVE MS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterised by demyelination and axonal degeneration. The molecular mechanisms that underpin axonal degeneration are relatively unexplored in MS. Studies using the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), have shown that the collapsin response mediator protein 2 (CRMP-2), an important tubulin-associated protein which regulates axonal growth, is phosphorylated during the neurodegenerative phase of the inflammatory disease. **Purpose:** The aim of this study was to determine whether pCRMP-2 was expressed during the neurodegenerative phase of MS. **Methods:** Human CNS tissue was obtained through the Department of Anatomical Pathology, The Royal Melbourne Hospital, in accordance with the NHMRC guidelines and the Monash University Human Research Ethics Committee for the use of human specimens in medical research. Post-mortem delay did not exceed 24 h and biopsy delay was < 1 h. The following tissues were used in the current study: MS CASES - 4 Chronic-progressive MS cases (67 plaques analysed from brain and spinal cord), 3 Acute-progressive MS cases (3 plaques analysed from brain); OTHER NEUROLOGICAL DISEASES – epilepsy (1 case from temporal lobe), Progressive Multifocal Leukoencephalopathy (PML, 3 cases from brain), Alzheimer's disease (AD, 2 cases from frontal lobe), Meningitis (1 case from frontal lobe); NON-NEUROLOGICAL DISEASES – death by natural causes (10 cases from brain). **Results:** The phosphorylated form of CRMP-2 (pThr555CRMP-2; the rho kinase specific site) is localised to spinal cord neuronal somata and axons in chronic-active grey and white matter MS lesions. The greatest level of pThr555CRMP-2 immunostaining was in AT8-positive degenerative axons and neuronal somata near infiltrating inflammatory cells and myelin debris. **Conclusion:** Our data suggest that pThr555CRMP-2 may be a surrogate marker of the neurodegenerative phase of axons and neuronal somata during MS lesions activity. This study correlates with our EAE data which suggest that pCRMP-2 may be a central mechanism that govern axonal degeneration during inflammatory demyelination of the CNS (as occurs in MS).

POS-MON-101

TAM RECEPTOR SIGNALLING IN INFLAMMATORY DEMYELINATION

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Background: Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). The TAM family of receptor tyrosine kinases (Tyro3, Axl and Mertk), and their ligands (Gas6 and Protein S (ProS)), have been implicated as important players during central demyelination, potentially regulating both neural and immune responses. We investigated the expression of TAM receptors and ligands in both the CNS and lymphocytes, in a model of inflammatory demyelination. **Methods:** Experimental autoimmune encephalomyelitis (EAE) was induced in C57Bl/6 mice (n=3-6 per group). Spinal cords, T-cells and B-cells were collected at 8, 15 and 21 days post-induction, as well as from healthy mice (n=6-8). Using quantitative PCR, we examined the expression of TAM receptor and ligand genes. **Results:** We found regulation of the expression of TAM receptor and ligand genes in all cell types studied. In spinal cord, Axl, Mertk and ProS expression increased with disease progression ($p<0.001$), whilst Tyro3 and Gas6 expression decreased ($p<0.001$). In T-cells, we found increased ProS expression with disease progression ($p<0.001$), and a transient increase in Gas6 expression at day 8 post-induction. In B-cells, Axl, Mertk and Gas6 expression were all transiently increased at day 8 post-induction ($p<0.01$) before returning to baseline levels. **Conclusions:** Previous literature has suggested that TAM receptors are not expressed by lymphocytes under physiological conditions. This study shows TAM receptor expression may be induced with lymphocyte activation in the context of EAE. The regulation of TAM receptor expression in B-cells prior to clinical onset in EAE, suggests that TAM signalling may be important in regulation of the B-cell response during EAE pathogenesis.

POS-MON-102

[^{18}F]-FLUMAZENIL: A NOVEL PET RADIOTRACER FOR THE LOCALISATION OF DRUG RESISTANT FOCAL EPILEPSY

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Purpose: Previous studies suggest that [^{11}C]-flumazenil (FMZ) can more specifically localise the epileptogenic zone (EZ) in patients with medically refractory focal epilepsy. However, practical aspects of [^{11}C] and requirement for arterial blood sampling have limited its clinical application. This study assessed the utility of the novel PET radiotracer, [^{18}F]-FMZ, without arterial sampling, for the localisation of the EZ in patients with drug resistant temporal lobe epilepsy (TLE). **Methods:** Three subject groups were studied; healthy controls (n=17), TLE patients with hippocampal sclerosis on MRI (n=12) and TLE patients with normal MRI (n=19). A 60min dynamic [^{18}F]-FMZ-PET scan and an FDG-PET scan were acquired. Blinded visual assessment of static images was undertaken. Parametric images of binding potential (BP) were generated and region of interest (ROI) analysis and statistical parametric mapping (SPM) used to localise the EZ. **Results:** Visual assessment of static images showed [^{18}F]-FMZ-PET to have high specificity (94.1%) and positive predictive value (94.4%), and moderate sensitivity (54.8%), for the localisation of the EZ, with a more restricted abnormality when compared with FDG-PET. ROI and SPM analyses also demonstrated a more restricted abnormality, with specific reductions in ipsilateral hippocampal FMZ BP, compared with more widespread hypometabolism on FDG. **Conclusions:** This study demonstrates the practical utility of [^{18}F]-FMZ PET for the localisation of the EZ compared with FDG in the pre-surgical evaluation of drug resistant TLE. [^{18}F]-FMZ PET shows a more restricted region of abnormality than FDG-PET, suggesting that it may more specifically delineate the EZ.

POS-MON-103

SELECTIVE LOSS OF AMPA RECEPTOR SUBUNITS IN THE RETICULAR THALAMIC NUCLEUS OF THE EPILEPTIC STARGAZER MOUSE

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Purpose. Stargazin is the prototypical transmembrane AMPA receptor (R) regulatory protein (TARP), controlling AMPAR trafficking and function. Reduced expression of stargazin in the stargazer mutant mouse thus leads to loss of synaptic AMPARs and altered excitation, ultimately resulting in absence epilepsy and ataxia. However, as absence seizures are thought to arise from hyperexcitation in the thalamocortical networks, it is unclear how loss of AMPARs could lead to the epileptic phenotype in stargazers. Other workers have reported that postsynaptic currents are reduced in inhibitory reticular thalamic nucleus (RTN) neurons but not in excitatory relay neurons of the ventral posterior (VP) complex in the stargazer. Our goal was to investigate if the altered currents in RTN neurons are associated with a change in total or synaptic expression of AMPAR. Methods. Western blot analysis and quantitative immunogold cytochemistry, were used to analyse global and synaptic AMPAR subunit protein levels respectively in RTN and VP of male epileptic stargazer and non-epileptic control littermate pairs (n=5-7). Results. AMPAR GluA4 subunit levels were significantly reduced (total protein by 68% $p<0.001$; synaptic by 46% $P<0.05$) in the stargazer RTN. Conversely, overall AMPAR expression was unchanged in the VP. Conclusion. The findings of this study provide molecular and cellular evidence for a selective regional loss of AMPAR protein in RTN, which could account for the loss of function at inhibitory neuron synapses. This could contribute to the stargazer epileptic phenotype by leading to disinhibition within the thalamocortical network and thus hyperexcitability.

POS-MON-104

COMPARATIVE BEHAVIOURAL PHENOTYPE OF PILOCARPINE INDUCED SEIZURES IN JUVENILE AND ADULT C57BL6J MICE

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Systemic injection of the muscarinic agonist pilocarpine to rodents results in acute seizures and status epilepticus (SE), with the latter resulting in chronic histological changes and spontaneous seizures that models human temporal lobe epilepsy. As a prelude to characterising the mechanisms underlying this epileptogenesis, we measured the responses to systemic pilocarpine in young (4-5 week, n= 28) and older (8-11 week, n=16) male C57Black 6J mice. Behavioural responses were monitored for 3 hrs and scored using a seizure scale. All mice displayed a consistent, time and dose-dependent series of behaviours that begun with immobility and mild tremors, followed by body shaking, falling and rearing seizures. Juvenile mice showed a more moderate pilocarpine response, with only 4 out of 11 displaying a falling seizure. Adult mice were more sensitive to pilocarpine, with a phenotype that included jumping seizures. The continuous seizures that defined SE was observed in 4 out of 14 adult mice, and was never observed in juvenile mice. However, adult mice also showed a high mortality (6 out of 14), even when a gradually ramping up pilocarpine dose schedule was used. Our results indicate marked developmental differences in the response to pilocarpine, with sensitivity increasing from juvenile to adult, in contrast with results in other rodent species. The low incidence of SE and high mortality make the C57Bl6J mice poorly suited as a model for temporal lobe epilepsy using the pilocarpine model. Preliminary studies in a mixed background adult mice species indicate a higher incidence of SE in response to pilocarpine with a lower mortality rate.

POS-MON-105

THE EFFECTS OF PHENCYCLIDINE TREATMENT ON THE NMDAR-NRG1 SIGNALLING PATHWAY IN RATS

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Phencyclidine (PCP), an NMDA receptor antagonist, is the best known drug that can mimic the range of symptoms of schizophrenia in humans and animals. Genetic linkage and post-mortem studies strongly suggest a role for altered neuregulin1 (Nrg1)/erbB4 signalling in schizophrenia pathology. This study investigated the relationship between the NMDA receptor and Nrg1 signalling pathways following adult PCP treatment. **Purpose:** To determine if adult PCP treatment affects gene or protein expression of molecules associated with the NMDAR-Nrg1 signalling pathway in rats. **Methods:** Male and female adult rats were treated with PCP (10mg/kg) or saline for 14 days. Rats were sacrificed (n=5/ group) on drug withdrawal days 1, 3, 5, 7, 10 and 14. RT-qPCR array, Western blot and receptor binding autoradiography were employed to analyse the gene and protein expression in the prefrontal cortex and hippocampus. **Results:** PCP treated rats displayed an increase in Nrg1 mRNA expression at several withdrawal time-points (day 3 onwards) in both areas, though significant only in the hippocampus at day 5 (1.77 fold increase; $p<0.01$), which was accompanied by an increase in BDNF (1.84 fold increase; $p<0.01$). In terms of protein expression, Nrg1 and erbB4 tended to be decreased in PCP treated rats ($p>0.05$), while p-erbB4 displayed significant decreases on days 7 and 10 of withdrawal (23-27%; $p<0.05$) in the prefrontal cortex. PCP had no significant effect on NMDA receptor binding. **Conclusion:** Adult PCP treatment had minimal effect on NMDA receptor expression, however this treatment did alter Nrg1 and erbB4 expression and activation in a time- and brain region-specific manner.

POS-MON-106

EFFECTS OF OLANZAPINE AND BETAHISTINE ON SEROTONIN 5-HT2A RECEPTOR BINDING IN THE RAT BRAIN

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Olanzapine is widely used in treating multiple domains of schizophrenia symptoms through its binding profile to various neurotransmitter receptors including a high antagonistic affinity to serotonergic 5-HT_{2A} receptors (5-HT_{2A}R). On the other hand, antagonistic affinity of olanzapine to histamine H_1 receptors is a main indicator of its weight gain/obesity side-effects. Our previous studies have shown that co-treatment of olanzapine and betahistine (a H_1R agonist and H_3R antagonist) could reduce obesity induced by olanzapine. This study aimed to investigate whether this co-treatment affects 5-HT_{2A}R bindings. Methods: Female Sprague Dawley rats (n=6/group) were administered orally (t.i.d) with either olanzapine (1mg/kg), betahistine (2.7mg/kg), olanzapine plus betahistine (O+B), or vehicle (control) for 2-weeks. Quantitative autoradiographic methods were used to detect the density of [^{3}H]ketanserin binding to 5-HT_{2A}R using a β-Imager. Results: Compared to the controls, olanzapine significantly decreased 5-HT_{2A}R bindings in the prefrontal ($p<0.001$) and cingulate cortex ($p<0.001$), and tended to decrease 5-HT_{2A}R bindings ($p=0.08$) in the nucleus accumbens core. Similar binding density changes in these nuclei were also observed in the O+B co-treatment group. However, betahistine did not affect 5-HT_{2A}R bindings in these regions ($p>0.05$). Conclusion: Both sole olanzapine and O+B co-treatment had similar effects to decrease the 5-HT_{2A}R density in the nucleus accumbens, prefrontal and cingulated cortex. It confirmed the important role of 5-HT_{2A}R in efficacy of olanzapine, which is not influenced by the co-treatment. Therefore, betahistine co-treatment would be a suitable combination therapy to reduce olanzapine-induced weight gain side-effects without affecting its therapeutic efficacy in treating schizophrenia symptoms.

POS-MON-107

ASSESSING THE ANTIDEPRESSANT POTENTIAL OF NOVEL $P2X_7$ RECEPTOR ANTAGONISTS *IN VIVO*

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Purpose: Over the last two decades there has been increasing evidence of a strong relationship between depression and immunological dysfunction in depressed patients. Excessive secretion of cytokines, including interleukin-1β (IL-1β), is increasingly recognised as a potential cause of depression. The $P2X_7$R modulates the release of IL-1β in macrophages and microglia and is involved in inflammatory and neurodegenerative mechanisms. This suggests that the $P2X_7$R could play a role in the pathophysiology of depression and that blockade of the $P2X_7$R might result in antidepressant-like properties. Here, we evaluate 3 novel analogues (PSB183, PSB187 and SMW44) of the lead adamantane amide antagonist first reported by AstraZeneca in 2003 for tolerability and then antidepressant activity in an in vivo model of depression- the forced swim test (FST). **Methods:** Tolerability of the compounds was assessed over 48 h in single mice including weight, temperature and activity. The compounds were then injected (i.p) into larger groups of mice (n=15/ group) that were subjected to the FST for 3 consecutive days. **Results:** All compounds were tolerable at all tested doses (up to 20 mg/kg). One of the 3 compounds, PSB183, caused a significant reduction in immobility time during the third day of repeated testing in the FST, suggesting an antidepressant response. **Conclusions:** As the antidepressant phenotype of PSB183 was only revealed after repeated testing, the compound appears to act by increasing resilience to the effect of repeated stress. Further, the antidepressant effect induced by P2X7R antagonism indicates the potential of this approach as a novel treatment strategy for depression.

POS-MON-108

EXOGENOUS ALLOPREGNANOLONE NORMALISES DEPRESSIVE-LIKE BEHAVIOURS AND NEUROGENESIS IN SOCIALLY ISOLATED RATS

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Chronic stress has been implicated as a causal factor in depression, and is associated with neuroendocrine dysfunction and impaired hippocampal neurogenesis. Depressed patients exhibit reduced levels of the neurosteroid allopregnanolone (ALLO), which is involved in regulating neuroendocrine activity. We propose chronic stress decreases ALLO production leading to dysfunction of the neuroendocrine system and impairment of hippocampal neurogenesis. **Purpose:** To examine whether exogenous ALLO treatment can normalize depressive-like behaviours, BDNF levels and hippocampal neurogenesis in a rat model of chronic stress. **Methods:** Male Wistar rats were subjected to 6 weeks of social isolation (SI) before receiving 4 weeks treatment. Rats were assigned to group-housed control, SI-placebo or SI-ALLO (1mg/kg/day s.c.) treatment groups. Depressive-like behaviour was assessed using the novelty-suppressed feeding (NSF) test and forced-swim (FS) test prior to, and at the end of, treatment. Animals were injected with IdU and CldU to assess progenitor cell proliferation and survival, respectively. **Results:** SI-placebo animals spent significantly more time immobile in the FS test than group-housed animals ($P=0.002$), however ALLO-treated SI animals were normalized to group-housed ($P=0.09$). Performance of SI-ALLO animals was also restored to group-housed levels in the NSF test ($P=0.089$). BNDF levels were reduced in socially isolated animals compared to control ($P=0.033$), but ALLO treatment normalized BDNF to group-housed levels ($p=0.101$). While SI was associated with reduced neurogenesis, ALLO-treatment restored the proliferation and survival of hippocampal progenitor cells to normal levels when compared to GH animals. **Conclusions:** Administration of exogenous ALLO following SI-induced chronic stress normalized depressive-like behavior, BDNF expression, and hippocampal neurogenesis. ALLO may therefore provide a novel therapeutic target for the treatment of depression.

POS-MON-109

INVOLVEMENT OF MULTIPLE PATHWAYS IN THE PATHOGENESIS OF EARLY-ONSET PARKINSONISM CAUSED BY MUTATIONS IN ATP13A2

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Purpose: Kufor-Rakeb syndrome (KRS, MIM606693) is a form of autosomal recessive, juvenile or early-onset, levodopa-responsive parkinsonism that has been associated with mutations in *ATP13A2* (*PARK9*, MIM610513). We identified novel compound heterozygous mutations, c.3176T>G (p.L1059R) and c.3253delC (p.L1085WfsX1088), in two siblings of Asian descent with KRS and examined pathogenicity of the mutations using cell models. **Methods:** We cloned the mutant *ATP13A2* from the proband and transiently expressed it in COS7 cells to examine the pathogenic mechanisms causing KRS. We also established human olfactory neurosphere and fibroblast cultures from the patient and controls to validate our findings under physiological conditions. All experiments were repeated at least two times in triplicate and acquired data were statistically assessed using a two-tailed Student's *t* test or one-way ANOVA followed by *post-hoc* Tukey-Kramer multiple comparison test. **Results:** Localisation studies using confocal microscopy (n>10 for each genotype) revealed that wild-type ATP13A2 localised to Lysotracker-positive and LAMP2-positive lysosomes, but not LC3-positive autophagosomes, while both truncating and missense mutated ATP13A2 failed to localise to this target. Mutant ATP13A2 was found to be retained in the endoplasmic reticulum and analysis by Western blotting (n=3) suggested that both mutant proteins were degraded by the proteasomal, but not the lysosomal pathways. This was confirmed by real-time RT-PCR (n=3), which showed a significant increase in the expression of unfolded protein response-related genes in patient cells. ATP13A2 mRNA containing the c.3253delC mutation was found to be degraded by nonsense-mediated mRNA decay, which was protected by cycloheximide treatment (n=3). In addition, the c.3253delC mutation was also associated with an increase in alternative splicing of ATP13A2 mRNA. **Conclusions:** Our findings indicate that these novel ATP13A2 mutations are indeed pathogenic and support the notion that multiple pathways contribute to the aetiology of KRS-related parkinsonism.

POS-MON-110

MATERNAL LACTOFERRIN SUPPLEMENTATION REDUCES BRAIN IMPAIRMENTS IN IUGR OFFSPRING BY UP-REGULATED BDNF EXPRESSION AND BRAIN METABOLISM

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Background: Lactoferrin (Lf), an iron-binding glycoprotein containing terminal sialic acid residues, is secreted in milk. Lf is synthesized in brains of both humans suffering from Alzheimer's dementia, and in the mouse model of Parkinson's disease, suggesting that Lf may play a role in neurodegenerative diseases. Objective: To investigate the neuroprotective effects of Lf in rats following prenatal exposure to dexamethasone (DEX) by 1H-MRS and hippocampal gene expression profiling. Method: Intrauterine growth restriction (IUGR) in rat pups was induced by maternal exposure to DEX supplemented with Lf (1g/kg b.w./day) during gestation and lactation. Neurochemical profiles of hippocampus (HC) and cortex (C) were determined in vivo by 1H-MRS at 9.4 T, and selected gene expression in hippocampus at postnatal day 7. Results: DEX in the rat model showed a significant decrease in the concentration of NAA-NAAG and Glu-Gln, markers of neuronal integrity and function, in the HC and C. This confirmed the altered brain development after DEX exposure and revealed the potential protective effect of Lf given to gestational and lactating dams in the rat model of prenatal exposure to adverse conditions. DEX-induced transcriptional changes in BDNF and DMT1, normalized with maternal bLf supplementation. Conclusion: Maternal milk Lf supplementation has a protective effect for neurodevelopment delayed IUGR in the rat model.

POS-MON-111

DOES MATERNAL OBESITY EXACERBATE HYPOXIA INDUCED BRAIN INJURY IN OFFSPRING?

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Obesity has long been known to be associated with a host of health issues. In woman of childbearing age, obesity is associated with increases in maternal morbidity and the likelihood of birth related trauma. For instance the risk of hypoxic-ischaemic (HI) brain injury to the newborn increases in obese women, and may be exacerbated due to a greater underlying inflammatory response. Here we examined the effects of maternal obesity in a neonatal rat model of HI injury. Sprague-Dawley rat mothers were fed chow or high fat diet (HFD) for 10 weeks prior to mating. At postnatal day 7, HI pups were anaesthetised with 1.5% isofluorane and underwent unilateral common carotid artery ligation followed by 3 hours of 7% oxygen. Sham littermates had the same procedure without ligation and were exposed to room air. Brains were removed 5 days post-injury for histological analysis. In HI pups from both chow (n=11) and HFD (n=13) dams, a significant reduction in brain volume was observed (5.9±2.6%, $p<0.01$ and 12.4±7.5%, $p<0.05$ respectively versus sham, t-test). The HFD group appeared to have a greater loss of brain tissue compared to the chow group however this was not statistically significant. Immunohistochemical staining using cell specific markers for microglia (CD11b) and astrocytes (Glial Fibrillary Acidic Protein) showed greater activation of these cells in injured brain hemispheres, suggesting a greater inflammatory response after injury. While further investigation is necessary to confirm these observations, this study provides an insight into the possible deleterious effect of maternal obesity in rats on the extent of brain injury in their offspring.

POS-MON-112

GENERALISATION OF USE-DEPENDENT LEARNING CAN BE EXPLAINED BY ALTERED SYNAPTIC WEIGHTS OF TRAINED NEURONS IN A POPULATION CODING MODEL OF CORTICAL PROCESSING

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Purpose: Human motor learning involves both error-based corrections and a use-dependent component that biases subsequent movements towards those repeated during practice. In an isometric forearm aiming task, we tested for bias in force direction in multiple areas of the workspace in response to a single training direction, and determined whether this bias is represented in an extrinsic or muscle-based reference frame. **Methods**: Training involved 40 maximal-force ballistic contractions toward a single target. The effects of training were tested in a low-force task that required subjects to move a cursor from the centre of a two-dimensional display towards radial targets. **Results**: In experiment 1 (n=10), we found that aiming direction was broadly biased towards the training direction according to a function that was well fit by linear scaling of synaptic weightings in a population coding model of cortical processing according to firing rate during training. In experiment 2 (n=12), when both the aiming task and training were conducted in the same (neutral) forearm posture, there was bias for high-force, but not low-force training. When the training contractions were performed in a 90° pronated forearm posture, whereas the aiming task was performed in a neutral forearm posture, there was aiming bias toward the training direction defined in extrinsic space, even for targets where this resulted in errors away from the pulling direction of the trained muscles. **Conclusion**: The effects of use-dependent learning in isometric aiming generalise broadly to untrained movement directions, are represented in an extrinsic rather than muscle-based reference frame, and are facilitated by training involving strong neural drive.

POS-MON-113

RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION OF THE IPSILATERAL MOTOR CORTEX AFTER BALLISTIC TRAINING DEPEND ON STIMULUS INTENSITY

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Purpose: In previous studies, unilateral ballistic training either increased or decreased corticospinal excitability for the untrained opposite limb. The objective here was to investigate whether these discrepancies can be explained by methodological differences such as the intensity of transcranial magnetic stimulation used to probe excitability, or the timing of excitability testing after training. Methods: Motor evoked potentials (MEP) were elicited by stimulating the ipsilateral cortex at high-intensity (70% MEPmax) and low-intensity (20% MEPmax) at specific time-points after performance of 300 ballistic movements of the index finger. Results: In the experiment (n=18), we found that ballistic practice significantly facilitated MEP size for high-intensity stimuli, whereas responses to low intensity stimulation were variable. MEP sizes at individual time-points were not significantly facilitated until 4min after training, although there was no difference between early and late responses when grouped over multiple time-points. Conclusions: The reliability of changes in corticospinal excitability after ballistic practice is dependent upon stimulus intensity. Time course effects in the untrained limb are more subtle that previously reported for the trained limb after high force contractions. Therefore, previous discrepancies in the ipsilateral corticospinal responses to ballistic training probably depend on experimental factors such as locus of attention and availability of visual feedback rather than the specific procedures used to assess corticospinal excitability.

POS-MON-114

ADAPTATION TO A NON-LINEAR VISUOMOTOR ROTATION OCCURS IN AN EXTRINSIC COORDINATE FRAME

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Purpose: When visual feedback about movements is rotated, people learn to adjust their motor outputs to achieve task goals in the new visuomotor conditions. This recalibration seems to occur in extrinsic spatial coordinates, as generalisation of prior learning occurs towards targets to similar locations in external space even with the use of muscles that were not trained. However, adaptations to altered limb dynamics generalise in an intrinsic reference frame. It is possible that the alterations in muscle activity for a given limb trajectory required in new dynamic conditions results in neural adaptation at more motor stages of the sensorimotor transformation. Here we applied a non-linear visuomotor perturbation that required altered muscle activation for a given cursor trajectory to re-assess whether aspects of visuomotor adaptation are coded in an intrinsic reference frame. **Methods:** Subjects (n=16) adapted to an anti-clockwise visual rotation, which was abrupt (n=8) or gradual (n=8) in onset, to reach a virtual target through the application of isometric wrist force. The magnitude of the perturbation was proportional to the instantaneous resultant force, resulting in a curved cursor trajectory for a linear force trajectory. Following adaptation, the forearm was rotated by 90deg and generalisation was assessed in the new posture to targets corresponding to the training direction in extrinsic and muscle space. **Results:** Subjects adapted to the perturbation by making curved movements, for both abrupt ($p<0.01$) and gradual ($p<0.01$) rotation conditions. Significant ($p<0.01$) generalisation was found towards the adapted target defined in extrinsic, but not muscle space. **Conclusion:** Visuomotor adaptation occurs predominantly in an extrinsic coordinate frame, even when altered muscle activation is required for a given movement trajectory.

POS-MON-115

META-ANALYSIS OF THE EFFICACY OF BASIC FIBROBLAST GROWTH FACTOR (BFGF) IN EXPERIMENTAL ISCHAEMIC STROKE

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Purpose Growth factors are hypothesized to support neuroprotection and enhance neuro-regeneration in models of focal cerebral ischaemia. Basic fibroblast growth factor (bFGF) has shown a potent trophic effect on brain neurons, glia and endothelial cells in preclinical studies. Here we undertake a systematic review and meta-analysis of bFGF in experimental ischaemic stroke. **Methods** Systematic review and DerSimonian and Laird random effects meta-analysis of studies describing the effect of bFGF in animal models of focal cerebral ischaemia where outcome was measured as infarct size or neurobehavioural score. Study quality was scored against a checklist. Stratified meta-analysis was used to assess the impact of study design characteristics on outcome. The significance level was set to $p<0.001$. **Results** Systematic review identified 21 publications of which 20 report infarct size (n=520) and 10 report neurobehavioural score (n=223). bFGF reduced infarct size by 25.7% (95% CI 21.7-29.8%) and improved neurological score by 28.1% (23.0-33.2%). Efficacy was higher with intra-arterial administration, and lower in aged animals with only 4.7% reduction of infarct size. A dose response relationship was seen for neurobehavioural score. Median quality score was 6/11 (IQR 5-7). The reported use of animals with associated co morbidities was rare, with no animals with diabetes or hypertension. **Conclusion** bFGF appear to have efficacy in experimental ischaemic stroke. However, further research is needed to test the efficacy in animals with co morbidities similar those in patients with stroke.

POS-MON-116

POS-TUE-001

EXOGENOUS LIF UPREGULATES EXPRESSION OF INHIBITOR OF APOPTOSIS MOLECULES IN NEURONS

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Traumatic injury to the central nervous system (CNS) is followed by the death of parenchymal CNS cells including neurons and oligodendrocytes. These cells continue to die by apoptotic mechanisms during the prolonged secondary phase of injury. It has been demonstrated that daily systemic administration of leukemia inhibitory factor (LIF) rescues oligodendrocytes from apoptotis following hemisection spinal cord injury in the mouse. This finding was associated with an increase in phosphorylation of Akt, and STAT-3, and upregulation of the cellular inhibitor of apoptosis protein-2 (cIAP2) in spinal cord lysates as demonstrated by western blot analysis and in mature oligodendrocytes as demonstrated by immunohistochemistry. Here we demonstrate upregulation of cIAP2 and also the X-Chromosome related IAP (XIAP) in differentiated human SHSY5Y neurons. This finding was also associated with downregulation of Caspase-3 expression in these neurons. These findings were not only reversed by the use of a neutralising anti-LIF antibody, but the use of this antibody caused significant death of these neurons. Therefore, LIF may play a prosurvival role in SHSY5Y neurons through upregulation of IAP molecules. These findings may have implications in neuronal trophic support strategies for treatment of spinal cord and brain injury in humans.

POS-TUE-002

ADENO-ASSOCIATED VIRAL MEDIATED DELIVERY OF PEPTIDE AGONISTS INTO THE RAT BRAIN

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PURPOSE: Relaxin-3 is a neuropeptide, that binds relaxin family peptide receptor 3 (RXFP3) expressing neurons, in limbic and hypothalamic areas. Relaxin-3/RXFP3 signaling has been implicated in metabolism, reproduction and stress in acute studies using a selective peptide agonist (R3/I5). This study was designed to develop recombinant adeno-associated viruses (rAAV) to chronically produce R3/I5 within RXFP3-rich brain areas of rats and assess the behavioural impact. **METHODS**: R3/I5 was cloned into the viral vector, CB-TR-FIB, which facilitates constitutive peptide secretion. Secretion of bioactive peptide was confirmed by transfection of HEK293T cells with FIB-R3/I5 and analysis of media by specific immunoassay and RXFP3 activity assays (n>3). Vectors (expressing GFP or FIB-R3/I5) were packaged into mosaic serotype 1/2 capsid to produce rAAV1/2 which was bilaterally infused into the hypothalamus of Sprague Dawley rats (n=8, per group). Feeding and bodyweight was monitored daily. **RESULTS**: Chronic expression of rAAV1/2-FIB-R3/I5 in hypothalamic neurons led to an increase in daily body weight and food intake, compared to control animals over the 7 week paradigm. There was no difference in locomotor activity between groups. However, in the light-dark box anxiety test the R3/I5 rats spent less time in the light side of the box ($P<0.05$). **CONCLUSION**: rAAV-FIB-R3/I5 mediated chronic RXFP3 activation in the hypothalamus leads to sustained altered feeding and body weight in rats. We will now utilize this tool to yield further insight into the role of relaxin-3/RXFP3 signaling in other regions of the brain.

POS-TUE-003

IN VITRO BIOACTIVITY CHARACTERISATION OF A SHUTTLE VECTOR CONTAINING THE GENE SEQUENCE FOR BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

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Purpose: This study is part of a gene therapy scenario aiming to use a viral vector containing the gene sequence for BDNF to encourage spinal axotomised fibres to reconnect with deafferented motor neurons. However, before the viral production can begin, the bioactivity of the BDNF-shuttle vector needs to be assessed. **Methods:** SH-SY5Y cells were treated with retinoic acid (RA) for 5 days to induce tyrosine kinase B (TrkB) receptors on their cell membrane. In parallel, Cos cells were transfected with the BDNF vector. BDNF secreted into the media surrounding the Cos cells was then transferred onto the RA-treated SH-SY5Y cells for 48 hours. SH-SY5Y cell controls included no treatment, RA treatment alone and BDNF-conditioned media exposure alone. The SH-SY5Y cells were then fixed, subjected to immunohistochemistry with an antibody to reveal the presence of BDNF into the cells, counterstained and then analysed under epifluorescence. **Results:** BDNF expression in Cos cells was confirmed by direct visualisation of the green fluorescent reporter protein. Immunohistochemistry showed that BDNF was internalised only by the SH-SY5Y cells after combined RA treatment and BDNF exposure. Moreover, only the SH-SY5Y cells treated with RA and exposed to the BDNF-rich media exhibited distinctive changes in morphology, that include robust neurite-like outgrowths. **Conclusion:** Cos cells transfected with the BDNF shuttle vector expressed and secreted BDNF. BDNF present in the Cos cell media was internalised by RA-treated SH-SY5Y cells, in all likelihood through its coupling with active TrkB receptors. This study therefore confirms that the BDNF shuttle vector is bioactive and suitable for viral-mediated gene therapy.

POS-TUE-004

DEVELOPMENT OF MATURE BDNF-SPECIFIC ANTIBODY AND SANDWICH ELISA SYSTEM

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Purpose BDNF (Brain-derived neurotropic factor) plays a crucial role in nervous system. Recent studies have shown that BDNF level in serum is related to psychiatric and other aging-related chronic diseases, but the ELISA (Enzyme Linked Immunosorbent 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 was to characterize mature BDNF-specific antibodies and set up ELISA system to measure BDNF level in samples. **Methods** 5 polyclonal or monoclonal antibodies against mature BDNF were purified and characterized by Western blot and ELISA using mature BDNF and proBDNF as antigens. To test specificity, NT3, NT4 and NGF were used on ELISA. Sandwich ELISA system was set up with capture and detecting antibody and specificity and sensitivity was tested. **Results** Out of 3 monoclonal antibodies to mature BDNF, two showed mature BDNF-specific reactivity on Western blot and ELISA. Interestingly, sheep anti-mature BDNF polyclonal antibody showed very specific reactivity only against mature BDNF, but not proBDNF on both of Western blot and ELISA. On the cross-reactivity test, sheep anti-mBDNF, B34D10 and B19-2-C2 showed high specificity against mBDNF only. B34D10/sh a-mature BDNF-biotin pair generated a straight standard curve and the detection limit was 100-200 pg/ml. **Conclusion** This study showed anti-mature BDNF antibodies were developed and they were highly specific only against mature BDNF, but not proBDNF, NT3, NT4 and NGF. ELISA system to quantify BDNF level was set up and will be used in further study.

POS-TUE-005

MAPPING OF THE INTERACTION SITE BETWEEN SORTILIN AND THE P75 NEUROTROPHIN RECEPTOR

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Neurotrophins (NT) comprise a group of neuronal growth factors that are essential to development and maintenance of nervous system. However, NTs can also be released in an immature form denoted pro-neurotrophins (proNT) with distinct and often opposing biological activities to that of their mature counterparts. In particular, they efficiently induce apoptosis during development and following spinal cord injury by engaging a receptor complex that comprises a heterodimer between $p75^{NTR}$ and Sortilin. **Purpose and methods:** Aimed at identifying the interaction domains in Sortilin and $p75^{NTR}$ we analysed binding between chimeric receptor constructs and truncated $p75^{NTR}$ variants by co-immunoprecipitation, surface plasmon resonance analysis, and Forster resonance energy transfer. **Results:** We found that the heterodimerization between Sortilin and $p75^{NTR}$ relays on contact points in both the extracellular and transmembrane domains of the receptors (n=5). We also found that the interaction critically depends on in the extracellular juxtamembrane domain of $p75^{NTR}$ (n=5). **Conclusion:** Although the intracellular domain of Sortilin does not partake in $p75^{NTR}$ binding, it regulates the rates of $p75^{NTR}$ cleavage by α- and β-secretase (n=6) and is required to mediate proNT stimulated cell death (n=5).

POS-TUE-006

INHIBITION OF UBIQUITIN-DEPENDENT PROTEASOMAL AND AUTOPHAGIC PATHWAYS PROMOTES ALS-LINKED SUPEROXIDE DISMUTASE 1 (SOD1) AGGREGATION IN A MOTORNEURON CELL LINE

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Purpose: Mutations in superoxide dismutase-1 (SOD1) are the most common genetic cause of familial amyotrophic lateral sclerosis (ALS), causing abnormal folding of SOD1 protein and aggregate formation. SOD1 aggregates co-localize with ubiquitin (Ub) forming ubiquitinated protein inclusions in affected motor neurons, a common hallmark of ALS pathology. The accumulation of SOD1-Ub inclusions suggests disruption to Ub-dependent degradation pathway(s), thus we hypothesize that genetically manipulating these pathways in cells will alter SOD1 aggregation and SOD1-Ub inclusion formation. Methods: NSC34 cells were transiently or stably transfected with a series of RFP-Ub expression constructs encoding wild-type (WT), monoubiquitin, polyubiquitin or dominant-negative forms of human Ub to target proteins to either lysosomes (K63, K48R), multivesicular bodies (K0) (collectively the endosome-lysosome system, ELS) or Ub-proteasome system (UPS) (K48, K63R). Ub expression and Ub-positive inclusion counts were examined using western blotting and immunocytochemistry (n=3). In addition, NSC-34 cells were co-transfected with RFP-Ub and mutant SOD1-EGFP A4V (mSOD1) to investigate the effect of ubiquitination pathways on SOD1 inclusion formation propensity, morphology and protein expression level (n=3). Results: Expression of all Ub constructs triggered spontaneous cytoplasmic Ub inclusion formation. Cells expressing Ub mutants targeting the ELS (K63, K48R, K0) showed a significant increase in Ub inclusion burden ($P<0.01$), suggesting that this pathway is instrumental in aggregate formation. Mono- or polyubiquitination of mSOD1 suppressed its aggregation, while expression of dominant-negative mutants (K48R, K63R) exacerbated mSOD1 inclusion formation ($P<0.01$). Conclusion: These data indicate a joint contribution from ubiquitination-driven ELS and UPS pathways in mSOD1 inclusion formation, highlighting these pathways as potential targets to combat SOD1 aggregation in ALS.

POS-TUE-007

NEUROTROPHIN RECEPTOR P75 AS A BIOMARKER FOR MOTOR NEURON DISEASE

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Purpose: An important step in finding effective treatments for motor neuron disease (MND) is to identify biomarkers that assess the effectiveness of potential therapies that could improve outcomes for this devastating disease. We have previously found that extracellular neurotrophin receptor p75 (p75NTR) is detectable by Immunoprecipitation/ Western blot (IP/WB) in urine from the mouse model of MND (SOD1G93A mice) well before symptoms appear but not in aged-matched controls[1]. We now wish to quantify urinary p75NTR in human MND and SOD1G93A mice. **Methods:** Quantitative ELISAs were developed to detect mouse and human p75NTR in the pg/ml range. Urine was collected from SOD1G93A and B6 aged-matched control mice and from human sporadic MND patients and controls. **Results:** Using a sandwich ELISA for mouse p75NTR, end-stage SOD1G93A mouse urine had 10.1 ± 1.2 ng/ml and aged matched B6 controls 3.2 ± 0.9 ng/ml (n=3). p75NTR in urine samples from SOD1G93A mice and controls are currently being quantified over life stages. Humans with early to mid stage MND have nearly 20 fold more p75NTR (3.8 ± 0.9 ng/ml) than controls (0.2 ± 0.01 ng/ml) p=0.005; n=5. **Conclusion:** Urinary p75NTR shows promise as a biomarker for MND in SOD1G93A mice and human sporadic MND. Further work is ongoing to correlate p75NTR levels with disease progression so that it can be used in clinical trials to monitor effectiveness of therapies. **References** 1. Biomarker for Motor Neuron Disease. Patent Application No 2011900312. Flinders University, Australia.

POS-TUE-008

TECHNIQUES FOR DETECTING AND VISUALIZING P75 NEUROTROPHIN RECEPTOR CLEAVAGE FRAGMENTS

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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. $p75^{NTR}$ is expressed widely during development, although expression decreases dramatically by adulthood. One of the cortical regions where expression is maintained is the cholinergic basal forebrain (CBF) neurons, whose loss is a hallmark of Alzheimer's Disease. $p75^{NTR}$ undergoes functional proteolysis generating a membrane bound C-terminal fragment (CTF) and a non-membrane-bound intracellular domain (ICD). However, there is conflicting evidence as to which cleavage fragment induces cell death. Lack of high quality antibodies and screening assays with the ability to detect the small amounts of $p75^{NTR}$ cleavage fragments that are endogenously expressed in the CBF makes further research into these mechanisms increasingly difficult. In the current study, we tested two novel ways of detecting and visualizing $p75^{NTR}$ cleavage. 1) A CTF specific antibody used to visualize this fragment in immunocytochemistry studies and 2) a screening assay for quantifying amounts of ICD present in cell lysates. Both techniques were tested for their abilities to measure cleavage fragments in cells under conditions known to induce $p75^{NTR}$ processing.

POS-TUE-009

UNRAVELLING THE ROLE OF USP9X PROTEIN MOTIFS IN NEURAL PROGENITOR POLARITY

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Purpose: The deubiquitylating enzyme, Usp9x, is a strong candidate to play a role in neural progenitor cell polarity. It is highly expressed in neural progenitors and many of its substrates include cell adhesion, trafficking and polarity components known to play a role in polarity. This study aims to investigate the role of Usp9x in neural progenitor polarity, using an in vitro overexpression system. Furthermore, a role for the various Usp9X protein motifs in cell polarity will be investigated. Specifically, the Fat LD, Clathrin box and EH1 motifs of Usp9x will be assessed, which are involved in regulation of protein trafficking and cell-cell adhesion. **Methods:** Usp9x was overexpressed in mouse embryonic stem cell derived neural progenitors, under the control of a Nestin promoter. Overexpression of Usp9X was found to promote self renewal of neural progenitors. This in vitro overexpression phenotype will be used as an assay to investigate a putative role for various Usp9X domains in cell polarity. Point mutations to various motifs will be introduced using site directed mutagenesis, to develop Usp9X overexpression constructs carrying various Usp9X domain-specific mutations. **Results:** Usp9x overexpression caused >80% of the neural progenitors to form highly polarised radial clusters when compared to empty-vector control cell lines, suggesting Usp9X promotes self-renewal. Mutations to various Usp9X motifs are proposed to either enhance or inhibit this polarity phenotype. **Conclusion:** Usp9x enhances the polarity and self-renewal of neural progenitors. Fat LD, Clathrin box and EH1 motifs are proposed to regulate the polarity of neural progenitor via their role in regulation of protein trafficking and cell-cell adhesion.

POS-TUE-010

DOWNREGULATION OF HDAC1 BY VALPROIC ACID IMPROVES VISUAL ACUITY IN ADULT VISUAL CORTEX

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Purpose: Neuronal circuits are shaped by sensory experience during critical periods (CP) of early postnatal brain development. We propose that changes in the external environmental can drive visual cortical plasticity during its CP through the epigenome. **Methods:** To understand the role of histone deacetylase 1 (HDAC1) in CP window formation, we extracted visual cortices from normal development and dark reared C57BL/6 mice for immunoblotting (n=4), qRT-PCR (n=6) and Chromatin ImmunoPrecipitation (ChIP) (n=3). To test if histone deacetylation limits plasticity in adulthood, we administered valproic acid (VPA), a HDAC inhibitor, in wild-type adult mice for 2 days. We performed biochemical assays such as miRNA and mRNA array profiling (n=4), immunoblotting (n=4) and ChIP (n=4). Visual water task (n=9) was carried out to assess the visual acuity of VPA-treated mice. **Results:** We report a developmental down-regulation of histone acetylation, regulated by HDAC1 in the mouse visual cortex. Dark rearing reduces HDAC1 expression in an experience-dependent manner. We disrupted the histone acetylation/deacetylation equilibrium with VPA, an antidepressant with HDAC inhibiting activity, in wild-type adult mice. VPA increases microRNAs expression which serves to reduce HDAC1 expression. VPA administration also improves visual acuity, as determined behaviorally. **Conclusion:** Taken together, our results suggest a potential clinical therapeutic application of VPA and HDAC1 for treating reduced visual acuity in adulthood.

POS-TUE-011

TROPOMYOSINS CONTROL NEURONAL MORPHOGENESIS AND THE MECHANICAL PROPERTIES OF NEURONAL CELLS

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Changes in cellular morphology and mechanic properties are dependent on the underlying actin cytoskeleton. Dynamic and structural properties of the actin cytoskeleton are regulated by a large pool of actin-associated proteins including tropomyosins, a family of actin-associated proteins controlling actin filament stiffness in an isoform dependent manner. In neurons products from three tropomyosin genes are found (TPM1, 3 and 4). Purpose: The current study aims to understand the role of TPM1 (TmBr1, TmBr2, TmBr3) and TPM4 (Tm4) gene products in early neuronal morphogenesis. Methods: Using B35 neuroblastoma cells overexpressing individual tropomyosin isoforms we investigated the impact of the actin cytoskeleton on neuronal morphology and early stages of neuritogenesis. To analyse the impact of tropomyosin overexpression on the mechanical properties of differentiating B35 cells we used Atomic Force Mircoscopy. Results: We show that the overexpression of tropomyosins is sufficient to induce the formation of neurites which is associated with an upregulation of the neuronal differentiation marker MAP2c. Tropomyosins differentially control the branching of neurites in db cAMP stimulated B35 cells. While TmBr3 and Tm4 increase the degree of branching, TmBr1 and TmBr2 did not promote the formation of neurite branches. The increase of branching in TmBr3 and Tm4 overexpressing B35 cells is associated with an increase in growth cone size and number of filopodia along these neurites. Furthermore we show that increased expression of tropomyosin impacts on the mechanical properties of differentiating neuroblastoma cells in an isoform specific manner with TmBr3 decreasing cell surface elasticity and increasing deformability. Conclusion: Our data suggest a central role for the actin-associated protein tropomyosin in neuronal differentiation and establishing of complex neuronal networks.

POS-TUE-012

DEVELOPMENTAL CHANGES IN EXPRESSION OF KCC2 AND NKCC1 IN NORMAL AND HYPOXIC-SCHEMIC NEONATAL PIGLET BRAIN

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Background: In the neonatal brain over activation of excitatory neurotransmitter systems secondary to hypoxic ischaemia (HI) causes significant neuronal cell damage and seizure generation. These neurotransmitter systems include (but are not limited to) the GABA receptor, and the chloride (Cl-) co-transporters NKCC1 and KCC2 The principle function of the GABA system is inhibition in the adult brain whilst in the neonatal brain GABA provides much of the excitatory drive. It is thought that during early postnatal development changes in NKCC1 and KCC2 expression drive the switch in function of the GABA system from excitation to inhibition through regulation of the intracellular concentration of Cl-. Thus administration of GABA enhancing drugs (antiepileptic), that in adult brain promote inhibition, may in neonates potentially worsen HI brain injury. NKCC1 and KCC2 have also been suggested to be involved in ischemia induced cell swelling, BBB breakdown, cerebral oedema and neurotoxicity. **Object:**To characterisethe normal developmental expression of NKCC1 and KCC2 in 4 cortical brain regions of neonatal piglets around the time of birth. To compare expression of NKCC1 and KCC2 in neonatal piglets following a hypoxic ischemic insult. **Methods:** For the developmental expression studies Piglets were obtained at several gestational time-points (Premature -23, -17, -14, -10, birth and one week old) by caesarean section or spontaneous vaginal delivery. For the HI experiments newborn piglets were placed through our established HI animal model. Brain tissues from all 4 cortical regions were collected and prepared for Western Blotting. KCC2 and NKCC1 protein expression was assessed using specific antibodies and visualised in different brain regions using ECL and measured by densitometry.**Result and Conclusion:** There were clear temporal and regional differences in developmental expression of KCC2 compared with relatively stable expression of NKCC1 in all cortices. There was marked upregulation of KCC2 around birth which may constitute the 'switch' in GABAA function; this was different between regions. Changes in expression of the KCC2 and to a lesser extent the NKCC1 co-transporter may play an important role in HI brain injury and seizures.

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POS-TUE-013

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 3 EXPRESSION IN THE DEVELOPING RAT FOREBRAIN

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The VEGF family, reported to have neuroproliferative and neuroprotective effects, acts via receptors VEGFR1-3. VEGFR3 has been demonstrated to be **expressed** in brain from early in gestation by neural progenitors, oligodendroglia and neurones and regulates neurogenesis in the subventricular zone (SVZ). The expression of VEGFR3 protein has not been comprehensively reported in developing CNS. **Purpose:** We examined cell-type specific expression of VEGFR3 in developing rat forebrain by immunohistochemistry, and in neurospheres generated from SVZ. **Methods:** Brains were collected and fixed from embryos (E13, E16, E18) and neonates (P2, P7, P15, P23). Double-labelling immunohistochemistry for VEGFR3 was performed with nestin, GFAP, NeuN, BT-III, NG2, and nNOS using ALEXA secondaries (n=2/age). SVZ tissue was collected from P2 neonates and neurospheres generated in vitro. Similar characterisation using double-labelling immunocytochemistry was performed. **Results:** VEGFR3 was expressed from E13 in the ventricular zone (VZ) and by E16 extended along radial glia, associated with nestin (neural progenitor marker) and nNOS (neuronal marker). Postnatally, VEGFR3 declined in VZ, SVZ and RMS and co-localised with nestin and GFAP (astrocytic marker), while increasing cortical and hippocampal expression was seen associated with NeuN, nNOS and BT-III (neuronal markers) and GFAP-positive astrocytes. Scattered cells co-expressed VEGFR3 and NG2 (oligodendroglial marker). In vitro, VEGFR3 was expressed by proliferating progenitors within spheres, their nestin expressing progeny, and by GFAP-positive astrocytes. Much rarer cells, of neuronal and oligodendroglial lineages, double-labelled with VEGFR3. **Conclusion:** We demonstrated broad expression of VEGFR3 protein across neural lineages. The early, persistent association of VEGFR3 with progenitors in proliferative regions of developing forebrain is consistent with an ongoing influence on neural progenitors, but intense expression later in astrocytic and neuronal lineages suggests an extensive function.

POS-TUE-014

HOMER3 REGULATION OF GENE TRANSCRIPTION IN NEURONS

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Homer proteins are known as cytosolic scaffold proteins, facilitators of cell signaling at the dendritic post-synaptic density. However, in immune cells, Homer proteins have also been shown to regulate gene transcription. The mechanisms that regulate gene transcription in neurons are poorly understood. We used a luciferase-reporter assay to determine whether Homer3 was a regulator of the transcription factor Nuclear Factor of Activated T cells (NFAT) in neurons. We found that a reduction in Homer3 expression levels increased NFAT-induced transcription to 150 ± 5% (n=3) of control levels. In contrast, overexpression of Homer3 reduced the level of NFAT-induced transcription to 45 ± 2% (n=3) of control levels, demonstrating that Homer3 is a negative regulator of NFAT in neurons. The L-type-voltage gated calcium channel (L-VGCC) agonist BayK8644 stimulated NFAT-induced transcription to 181% ± 13% of control Luciferase expression (n=6). Paradoxically we found that Homer3 expression was necessary for L-VGCC activation of NFAT-induced transcription. Reducing Homer3 expression prior to L-VGCC activation reduced NFAT-induced transcription levels to 137% ± 5% of control levels (n=9). The data suggest multiple roles for Homer 3 in the regulation of NFAT-induced transcription. Known neuronal gene targets of NFAT include BDNF, GluR2 and the IP3R, all vital to neuronal function. BACE1, an important enzyme in the pathogenesis of Alzheimer's disease is also a target of NFAT and we have found that a reduction in Homer3 expression increases the production of BACE1. Our data support a novel mechanism for the regulation of NFAT-induced transcription in neurons, with important implications for neuronal function.

POS-TUE-015

TRANSCRIPTIONAL CROSS-REGULATION BY *NFI* FAMILY MEMBERS IN DEVELOPING BRAIN

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The *Nuclear Factor One* (*Nfi*) transcription factors play important roles in brain development. *Nfia* and *Nfib* are highly homologous, and are both expressed during neuronal and glial differentiation. *Nfia*$^{-/-}$ and *Nfib*$^{-/-}$ mice display phenotypic similarities and differences. Although single heterozygous mice did not display a phenotype, double-heterozygous animals display brain defects. This suggests that these genes may cooperate in their regulation of downstream targets and may regulate the transcription of each other to provide a specific level of Nfi protein required for its function. **Purpose:** To investigate whether Nfib cross-regulates *Nfia* in brain development. **Methods:** RNA was isolated from E16 neocortex and hippocampus of wild type and *Nfib*$^{-/-}$ mice. *Nfia* expression was analysed by expression profiling and quantitative PCR. Analysis of Nfia expression by immunohistochemistry on knockout brains was also performed. **Results:** A comparison of expression profiles of neocortex (n=3) and hippocampus (n=3) from *Nfib*$^{-/-}$ mice revealed an up-regulation of *Nfia* compared to wild type ($p \leq 0.05$ and $p \leq 0.001$, Student's t-test). These data were confirmed by qPCR. Immunohistochemical staining of Nfib brains displayed increased expression of homozygous mice (n=3) compared to heterozygous or wild type (n=8). **Conclusion:** In E16 neocortex and hippocampus, *Nfia* is up-regulated in *Nfib*$^{-/-}$ mice, both on mRNA and protein levels. Thus, Nfib may repress *Nfia* expression in vivo.

POS-TUE-016

DIFFERENTIAL EXPRESSION PATTERN OF MIR-29B IN DORSAL ROOT GANGLIA FROM SPHINGOSINE KINASE DEFICIENT MICE

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Purpose: MicroRNAs (miRNAs) are small non-coding RNAs which are able to induce degradation of mRNA and inhibit translation of proteins. Their role in the function of nociceptive neurons remains is unknown. Since a given miRNA regulates expression of multiple target mRNAs, neuronal miRNAs provide an extremely powerful mechanism to dynamically adjust the protein content of neuronal compartments without requiring new gene transcription. **Methods & Results:** qPCR and In Situ Hybridisation showed miR-29b expression localized in nuclei of sensory dorsal root ganglion (DRG) neurons of wild-type mice (wt, C57/Bl6) in situ and in vitro (n = 3). We used qRT-PCR to determine miR-29b regulation in neurons in response to nerve injury (sciatic nerve transection, ScNT) and inflammation (CFA injection). miR-29b expression was unchanged in wt DRG in response to inflammation (n = 6) but significantly higher expressed (3.9fold) in ipsilateral DRG 6d after nerve damage (paired t-test, n = 5). Predicted target genes of miR-29b such as PTEN and Camk2 were lower expressed in the same samples (n = 3). MiR-29b was significantly lower expressed in lumbar DRG of sphingosine kinase1 (Sphk1) deficient mice (Sphk1$^{-/-}$) in comparison to wt mice. Reduced S1P synthesis in Sphk1$^{-/-}$ & Sphk2$^{-/-}$ neurons also modulated the miR-29b response to inflammation with significantly reduced expression in DRGs ipsilateral to the inflamed paw (paired t-test, n = 4). **Conclusion**: The expression of miR-29b changes in DRG neurons in response to nerve damage and inflammation and might be dependent on the presence of Sphk enzymes and the generation of S1P.

POS-TUE-017

INVESTIGATING THE MOLECULAR MECHANISMS OF MRF-MEDIATED REGULATION OF CNS MYELINATION

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Purpose: Whereas significant progress has been made in understanding the structure of myelinated axons, the mechanisms that regulate gene expression and morphology in myelinating oligodendrocytes remain incompletely understood. Recently, we identified a novel transcriptional regulator (Gene Model 98 or Myelin Gene Regulator Factor [MRF]) which is specifically expressed at the postmitotic/myelinating phase in oligodendrocytes, and is both necessary and sufficient to promote many aspects of myelination. Here we investigate the mechanism by which MRF promotes CNS myelination and the transcriptional networks that may underlie the myelination process. **Methods:** Chromatin-immunoprecipitation of MRF from cultured oligodendrocytes in conjunction with Illumina sequencing (ChIP-Seq) was used to identify putative MRF target sequences. In vitro Luciferase assays were used to confirm functional significance of MRF-bound regions and to investigate the relationship between MRF interacting partners at these enhancer regions. DuoLink in situ PLA technology was utilised to demonstrate direct protein-protein interactions. **Results:** Using ChIP-Seq we identified 2085 statistically significant peaks/MRF-bound active regions, which were strongly skewed towards genomic regions within or surrounding oligodendrocyte/myelin genes. Manual curation identified 79 active regions/peaks proximal to genes known to be regulated by MRF and/or implicated in myelination. Luciferase assays confirm that a number of these regions act as MRF-dependent transcriptional enhancers (n=3), including regions proximal to the MAG, MBP, PLP, Cntn2 and Tfn genes. This MRF-induced gene expression is significantly altered after overexpression or siRNA knockdown of Sox10 (n=3); and MRF and Sox10 were shown to interact physically (n=3). **Conclusion:** Together these data indicate that MRF binds to regulatory elements of myelin genes, establishing a direct role for MRF in the transcriptional regulation of genes that underpin oligodendrocyte maturation and CNS myelination.

POS-TUE-018

ACTIVATION OF SK CHANNELS IN SPINES AND DENDRITES BY ACTION POTENTIALS

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Rises in intracellular calcium are vital for a myriad of functions in neurons, ranging from ion channel regulation to gene expression. One of the important channel types modulated by changes in intracellular calcium is the small conductance calcium-activated potassium channel or SK channel. At the soma these channels are known to be involved in the medium afterhyperpolarisation after an action potential (AP), but their function during APs in other neuronal compartments is poorly understood. **Purpose:** To investigate the potential activation of SK channels in dendrites and spines during backpropagating APs. **Methods:** Whole-cell patch-clamp recordings were made from cortical layer 5 pyramidal neurons in brain slices from rats. Changes in intracellular calcium were monitored with a confocal microscope using a calcium-sensitive fluorescent dye introduced through the recording pipette. **Results:** Single APs produced an increase in intracellular calcium in basal dendrites and spines. Application of apamin to block SK channels led to an increase in calcium influx during backpropagating APs by 31.3 ± 7.8% in spines ($p<0.001$; n=26) and 17.1 ± 9.0 % in the adjacent dendrite ($p<0.001$; n=26), indicating that single backpropagating APs activate SK channels in spines and dendrites, possibly leading to modulation of the AP waveform. The effect of apamin was occluded by the R-type calcium channel inhibitor SNX 482 (n=21), suggesting functional coupling between SK channels and R-type calcium channels. **Conclusion:** In cortical pyramidal neurons SK channels in spines and dendrites are activated by backpropagating APs following calcium influx through R-type calcium channels. We hypothesise that SK channel activation during backpropagating APs may be important for regulating the induction of spike-timing dependent synaptic plasticity.

POS-TUE-019

ALTERTED HIPPOCAMPAL EXCITABILITY IN TWO MOUSE MODELS OF HUMAN FEBRILE SEIZURES

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Introduction: Febrile seizures (FS) affect ~3% of the population and have a clear genetic predisposition. We developed two mouse models based on human FS mutations; a voltage-gated sodium channel β1(C121W) mutation and a $GABA_A\gamma2$(R43Q) receptor mutation. **Purpose:** To determine basal network properties of these two models and determine their temperature dependence. **Methods:** *In vivo FS susceptibility:* C121W (n=28), R43Q (n=14) and WT (n=25) littermates were placed into a 41°C chamber and time to first tonic-clonic seizure measured. *In vitro extracellular electrophysiology:* Oscillations, induced by tetanic-stimulation of the CA1 stratum oriens, were recorded in the stratum pyramidale in slices from C121W (n=10), R43Q (n=6) and WT (n=17) mice and their power spectra, duration and delay to onset were determined. **Results:** *In vivo:* C121W and R43Q mice were significantly ($p<0.05$) more sensitive to thermally triggered seizures compared to littermate controls. *In vitro:* At 32°C oscillation duration was significantly ($p<0.05$) longer in C121W mice compared to controls with no difference seen in the R43Q model. At 32°C significant ($p<0.05$) differences in the power spectra were observed in both the C121W and R43Q mice as compared to controls suggesting changes in hippocampal network stability. **Conclusions:** Mice containing the C121W or R43Q mutation are more susceptible to thermally triggered seizures *in vivo*. Furthermore, both of these mutant models have altered hippocampal network excitability, which may contribute to their increased FS susceptibility.

POS-TUE-020

HOW DO LRPS AND THEIR LIGANDS AFFECT LTP IN THE HIPPOCAMPUS?

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Purpose: Low density lipoprotein receptor-related proteins (LRPs) are multifunctional receptors that bind a variety of ligands, including amyloid-beta and metallothionein, and interact with NMDA receptor and PSD-95. We hypothesised that LRPs are able to modulate long-term potentiation (LTP) in hippocampal neurons by a process dependent on the nature of the LRP ligand and its cargo. To examine this question, we established a hippocampal neuron culture model to identify expression and localisation of LRP receptors in these cells. We then examined the effect of metallothioneins, including zinc-binding and non-metallated forms, on LTP and neuronal activity. **Methods:** Mouse hippocampal neuron cultures were grown for 3, 7, 14 or 21 days (in each group n=3) and studied by immunocytochemistry or real-time polymerase chain reaction to identify expression and localisation of two LRPs, LRP1 and LRP2. LTP was measured in mouse hippocampal slices treated with amyloid-beta and either zinc-bound or non-metallated metallothionein (in each group n=3). **Results:** Expression of LRP1 and LRP2 was on the cell body and processes of hippocampal neurons at every time point. LRP1 also associated with synaptic markers. The distribution of LRPs did not alter with maturation. Amyloid-beta decreased LTP in hippocampal slices (193 ± 16 versus 135 ± 16). Non-metallated metallothionein effectively protected against amyloid-beta inhibition (177 ± 23 versus 135 ± 16) but zinc-bound metallothionein was less effective than this (146 ± 23 versus 177 ± 23). **Conclusion:** Hippocampal neurons in culture express LRP1 and LRP2 in cell bodies, processes and synapses, and distribution does not change during maturation. LRP ligands protect against amyloid-beta induced LTP inhibition, but results indicate that the cargo of the ligand, such as zinc bound to metallothionein, is important in modulating this effect. As LRPs bind a large variety of ligands, our results suggest a mechanism by which extra-neuronal factors modulate synaptic processes like LTP.

POS-TUE-021

EPISTATIC INTERACTIONS AT THE AXON INITIAL SEGMENT IN GENETIC EPILEPSY

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Purpose: Voltage-gated sodium channels have been identified as super culprit in genetic epilepsy. In neurons SCN2A and SCN8A channels are particularly enriched at the axon initial segment (AIS), a region critical for regulating excitability. While SCN2A is a known epilepsy gene, it was only last year that SCN8A was implicated, in an ENU- screen. A modifier was mapped to Scn2a, with more severe spike-wave discharges when the Scn8a^{8J} mutation was present in C3HeB/FeJ compared with C57BL/6J. The convergence of these two alleles at the AIS provides a potential mechanistic basis for this epistatic interaction. The aim was to characterise the Scn8a and Scn2a variants with the hypothesis that they would collectively determine AIS properties and subsequently seizure susceptibility. **Method**: Automated patch clamping using the Nanion patchliner, was used analyse mutants transiently transfected into HEK293T cells. **Results**: Analysis of the Scn2a modifier allele revealed hyperpolarising shift in the voltage-dependence of activation ($p<0.001$, N=22), and an increased recovery from inactivation ($p<0.05$, N=19), both major determinants of excitability. A small hyperpolarising shift in the voltage-dependence of inactivation ($p<0.01$, N=16) was seen that is unlikely to have a major impact on excitability. **Conclusion**: The Scn2a modifier allele is likely to impact excitability by decreasing the threshold for AP firing at the AIS potentially altering forward and backward propagation. Future analysis of Scn8a will reveal how the two mutants combine to exacerbate seizure phenotype. Such mechanisms are likely to be common in human epileptic syndromes where multiple genes are likely to be seen.

POS-TUE-022

ELECTRICAL COMPARTMENTALIZATION OF THE APICAL DENDRITIC TUFT OF LAYER 5 PYRAMIDAL NEURONS

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Purpose: The apical dendritic tuft of layer 5 pyramidal neurons receives substantial excitatory synaptic input through cortico-cortical circuitry. Imaging experiments from behaving rodents have shown that the apical dendritic tuft is highly electrically active. Despite this, little information is available on the electrical properties of the apical dendritic tuft of layer 5 pyramidal neurons **Methods:** Dual and triple whole-cell current-clamp recordings and two-photon calcium imaging experiments were made from rat layer 5 pyramidal neurons maintained in brain-slice preparations. **Results:** Simultaneous whole-cell recordings from the soma, nexus of the apical dendritic trunk, and sites within the apical dendritic tuft revealed that axonal action potentials decrementally spread from the soma to the nexus, and sharply attenuated as they invaded the apical dendritic tuft (n=20). Apical dendritic trunk spikes, evoked by the injection of current at the apical dendritic nexus, attenuated substantially into the apical dendritic tuft, in a distance-dependent manner (n=110). Two photon-calcium imaging showed that apical dendritic trunk spikes failed to invade terminal tuft dendrites. Consistent with this, intense tuft to nexus voltage attenuation was apparent for step voltage excursions and EPSP waveforms. Excitation of the apical dendritic tuft at sites close to or more distal from the branch point of the primary apical tuft dendrite however, evoked localized, TTX-sensitive, sodium spikes. Intense voltage attenuation however ensured that sodium spikes generated at secondary apical dendritic tuft sites did not evoke apical dendritic trunk spikes. Furthermore, multisite two photon glutamate uncaging revealed that dendritic electrogenesis could be evoked at sites throughout the apical dendritic tuft, but weakly spread to the apical dendritic trunk and failed to evoke trunk spikes or neuronal output. **Conclusions:** The apical dendritic tuft of layer 5 pyramidal neurons is electrically active, but intense voltage attenuation ensures electrical compartmentalization under *in vitro* conditions.

POS-TUE-023

CHOLINERGIC AND SEROTONERGIC CONTROL OF NEOCORTICAL LAYER 1 INHIBITORY INTERNEURONS

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Purpose: Neocortical activity is state-dependent and controlled by ascending neuromodulatory systems. As imaging studies have shown that the activity of the apical dendritic tuft of neocortical pyramidal neurons, which are confined to layer 1, is state-dependent. We investigated if layer 1 inhibitory interneurons were controlled by ascending neuromodulatory systems. **Methods**: Four classes of layer 1 interneuron were characterized in brain-slice preparations of the neocortex using whole-cell patch-clamp techniques and morphological reconstruction. Acetylcholine (100 µM) or 5-HT (50-100 µM) were rapidly applied (10-30 ms) to defined regions of the recorded cell by pressure application. **Results**: Acetylcholine depolarised all classes of layer 1 interneuron, by the activation of nicotinic receptors. In 20% of the cells acetylcholine evoked an action potential from the resting membrane potential. The effect of 5-HT were diverse: in 24% of classical accommodating cells (cACs) 5-HT depolarized the neuron via the ionotropic 5-HT3 receptor, while 43% of cACs showed a long-lasting hyperpolarisation, while the remaining cells showed a serried mixed response. In neurogliaform cells (NGFCs), 5-HT evoked a complex response, characterized by a long-lasting depolarization crowned by the appearance of a multiphasic hyperpolarisation. The multiphasic hypeprolarisation was reminiscent of inhibitory synaptic activity. This response was not sensitive to the sodium channel blocker TTX, but was generated with increased security when 5-HT was applied to the axonal arbor. **Conclusions**: Ascending activating systems powerfully control the membrane potential of layer 1 inhibitory interneurons. In NGFCs cells, which posses a dense axonal nest confined to layer 1 and act to globally inhibit pyramidal neurons, 5-HT leads to the generation of a novel form of inhibition, which is expressed autaptically and may be conveyed to postsynaptic pyramidal neurons.

POS-TUE-024

FUNCTIONAL RELEVANCE AND PHYSIOLOGICAL BASIS OF TARGET-SPECIFIC SHORT-TERM DEPRESSION IN THE LAYER IV EXCITATORY CIRCUIT

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Introduction: Using computational and experimental approaches, we investigated the functional role and physiological basis of the following target-specific short-term depression reported in layer IV of barrel cortex. Star pyramidal and pyramidal neurons show regular firing (class-1) and receive synapses that show release-independent depression and faster recovery at higher stimulus rates (type-2). Spiny stellate neurons show spike-frequency adaptation (class-2) and receive synaptic inputs, which show release-dependent depression and constant recovery (type-1). **Methods**: For simulations, Hodgkin-Huxley-based class-1 and -2 point model neurons were used, receiving either 500 type-1 or -2 synaptic inputs (α-function) with peak conductance of 1nS. Reliability of rate coding was measured using Fisher information (FI); a higher value indicates greater reliability. Experimental whole-cell recordings were done in 15-19 day-old rats at 35±1°C to estimate membrane time constants of neurons. **Results**: Class-1 neurons can reliably encode stimulus rate when receiving type-2 but not -1 inputs (FI an order to magnitude smaller). Class-2 neurons only encode a rate change reliably when receiving type-1 but not -2 synapses. Membrane time constants of star pyramidal and pyramidal neurons are twice those of spiny stellate neurons with values 56.2±2.5 (*n*=5), 64.5±5.3 (*n*=5) and 29.1±1.1 ms (*n*=3), respectively. **Conclusion**: Spiny stellate neurons are more suited to integrate EPSPs of rapidly depressing type-1 rather than those of quickly recovering type-2 synapses, which are more effectively integrated by star pyramidal or pyramidal neurons. Our results suggest that target-specific short-term depression in layer IV might facilitate reliable and specific neuronal encoding when pre- and postsynaptic properties are appropriately matched.

POS-TUE-025

TASK-LIKE POTASSIUM CHANNELS POWERFULLY CONTROL DENDRITIC ELECTROGENESIS IN LAYER 5 PYRAMIDAL NEURONS

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Purpose: In awake behaving rodents imaging approaches have shown the generation of long duration dendritic electrogenesis in the distal apical dendritic tree of layer 5 pyramidal neurons. In contrast, in anaesthetised animals or under brain-slice conditions, whole-cell recording techniques have revealed the generation of only discrete apical dendritic spikes at distal apical dendritic sites. These data suggest that modulatory systems substantially control dendritic electrogenesis. **Methods:** Dual and triple whole-cell current-clamp recordings and two-photon calcium imaging experiments were made from rat layer 5 pyramidal neurons maintained in brain-slice preparations. **Results:** Simultaneous whole-cell recordings from the soma and the nexus of the apical dendritic trunk were used to access integrative properties. The injection of incremental steps of positive current at the soma resulted in the generation of repetitive action potential (AP) firing. In contrast, a single, discrete, apical dendritic trunk spike was generated at the onset of positive current steps injected at the apical dendritic nexus across a wide current range (n=63). Bath application of barium (20-50μM), quinidine (25μM), bupivacaine (20μM) and muscarine (5μM), minimally altered somatic input-output relationship, but greatly prolonged dendritic electrogenesis, resulting in the generation of a large amplitude plateau potentials that evoked repetitive axonal AP firing. Plateau potentials were TTX-resistant, but blocked by the calcium channel antagonist nickel (250μM). Two-photon calcium imaging and voltage recording showed that quinidine significantly increased the spread of dendritic electrogenesis into the apical dendritic tuft. Furthermore, quinidine (25μM) dramatically facilitated the interaction between somatic AP firing and subthreshold dendritic synaptic input. **Conclusions:** This pharmacological profile indicates that TASK-like potassium channels powerfully control dendritic electrogenesis in layer 5 pyramidal neurons, and suggest that TASK channels are a key target for ascending cholinergic control of neocortical activity.

POS-TUE-026

A CHANNEL EXTENDING FROM THE CATALYTIC CLEFT OF TYROSINE HYDROXYLASE IS INVOLVED IN HIGH AFFINITY CATECHOLAMINE INHIBITION

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PURPOSE: Tyrosine hydroxylase (TH) controls the rate of production of the catecholamines, dopamine, noradrenaline and adrenaline. Catecholamines bind TH irreversibly and with high affinity to the active site iron and other active site residues to inhibit catalysis. Only phosphorylation of the N-terminus and subsequent conformational changes allow dissociation of bound catecholamine, thereby increasing enzyme activity. The mechanism for the absolute irreversibility of the high affinity catecholamine binding is currently unknown; the two existing TH crystal structures lack the N-terminal 156 residues. Despite this, a body of evidence suggests that interaction between key areas of the N-terminus and the catalytic domain traps catecholamine in the active site of TH until phosphorylation occurs. Using the TH crystal structure, we have focused on a channel extending out of the active site into which the N-terminus could fold and made substitutions of three amino acids lining the cleft, A297L, E362Q and E365Q. METHODS: Changes to the high affinity site were assessed by measuring TH activity of recombinant TH in the presence and absence of saturating dopamine (20μM) using the tritiated water release assay. Wild-type TH was included in all experiments. RESULTS: When dopamine was bound to the high affinity site, wild-type TH was inhibited to 13% of its activity in the absence of dopamine. For A297L, E362Q and E365Q the level of inhibition was dramatically reduced, with specific activities inhibited to 55%, 28% and 60% respectively (n=3 $p<0.05$). CONCLUSIONS: The results from this work indicate that inhibition through the high affinity site involves residues in this channel.

POS-TUE-027

CHARACTERIZATION OF CONNECTIONS BETWEEN THE INFRALIMBIC AND PRELIMBIC AREAS OF THE MEDIAL PREFRONTAL CORTEX

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Purpose: The acquisition and consolidation of fear is highly dependant on timed neuronal network activity that involves specific parts of the amygdala and higher cognitive prefrontal areas (Sotres-Bayon, 2010). The extinction of learned fear, which occurs when a conditioned stimulus is presented without the previously paired aversive cue, can be modulated by neuronal activity in the infralimbic region of the medial prefrontal cortex (ILPFC). However, activation of the prelimbic region (PLPFC), which is mainly involved in the acquisition of fear, has an opposite effect to the ILPFC does during fear extinction (Vidal-Gonzalez, 2006), enhancing the fear response. It's still not known if the PLPFC directly modulates amygdala neurons to alter fear responses during extinction learning or if these neurons modulate the output from the ILPFC. To investigate this we have begun to study the nature of connections between the infralimbic and prelimbic prefrontal cortex. **Methods:** To study connections between the ILPFC and PLPFC, we either (i) made injections of a retrograde tracer (beads) into the ILPFC to label neurons projecting to the ILPFC or (ii) Lentivirus/Adeno-associated-virus expressing the light-gated cation channel channelrhodopsin-2 (ChR2) into the PLPFC to activate inputs from the PFPLC. Whole-cell recording were then made from neurons in the ILPFC or PLPFC to understand the connections between these two brain regions. **Results:** Retrograde tracer injections revealed that PLPFC neurons from layer 2/3, layer 5 and layer 6 all project to the ILPFC (n=3). Equal numbers of neurons were found in all layers of the ILPFC. ChR2 infection of the ILPFC showed that there was a pronounced projection onto layer 5/6. This projection was glutamatergic as responses of PLPFC neurons to brief light pulses in animals that received ChR2-injections into the PLPFC were blocked by NBQX (n=4). **Conclusion:** The findings of this study point to an excitatory glutamatergic projection from the PLPFC to the ILPFC which might modulate ILPFC output during fear extinction learning in a timing dependant manner.

POS-TUE-028

$GABA_A$ RECEPTORS CONTAINING THE γ1 SUBUNIT OPEN IN THE ABSENCE OF AGONIST

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Purpose: $GABA_A$ receptors are pentameric chloride channels that open in response to the endogenous ligand GABA. Some of these receptors, such as the widely studied α1β2γ2 subtype, have also been reported to open in the absence of agonist. We are interested in whether the less-studied γ1 subunit confers agonist-independent opening probability, because this might contribute to a tonic inhibitory current sometimes seen in the central amygdala, where this subunit is highly expressed. **Methods:** HEK AD-293 cells were transfected with plasmid DNA for human $GABA_A$ receptor subunits, and GFP. For some experiments, cells were stably transfected. Cells expressing GFP (or stably transfected) were patch clamped using solutions of symmetrical chloride, and voltage clamped at -70mV. Ringers solution was continuously applied to the target cell using an array of fine tubes that could be moved to switch between drug-containing and control solution. Cells were first tested for receptor expression with saturating (1mM) GABA. After the GABA had washed off and the current returned to baseline, we applied the pore blocker picrotoxin at 100μM to assess the presence of endogenous current. Current changes induced by picrotoxin were normalised to the amplitude of the GABA response. **Results:** For α2β2γ2L receptors, mean picrotoxin-induced current amplitude was 0.024±0.09% of the saturating GABA amplitude (n=6). For α2β2γ2S it was 0.46±0.14% (n=7). However, for α2β2γ1 receptors, picrotoxin-induced current was much larger: 5.6±1.1% of the GABA induced current (n=11). The mean current amplitude for α2β2γ1 receptors was 10.97pA/pF, with a range of 0.04 to 43.77pA/pF. **Conclusion:** γ1-containing receptors display greater agonist-independent opening probability than γ2-containing channels. At high levels of expression, agonist-independent gating by these channels could contribute to tonic neuronal inhibition.

POS-TUE-029

SITES OF ACTION OF GHRELIN RECEPTOR LIGANDS IN CARDIOVASCULAR CONTROL

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Purpose: Current literature indicates that ghrelin receptors at different sites influence blood pressure in different ways: activation of peripheral receptors or receptors in the medulla oblongata lowers blood pressure, whereas receptors in the spinal cord mediate blood pressure rises. **Methods:** We used mice expressing enhanced green fluorescent protein (EGFP) under control of the promoter for GHSR and in situ hybridisation to localise receptors. Effects on rat and mouse blood vessels in vitro were measured by myography. Rat blood pressure and sympathetic nerve activity were measured in vivo. **Results:** Receptor was detected in neurons in the CNS by EGFP immunoreactivity and in situ hybridisation, but was not detected in blood vessels under the same conditions. Moreover, isolated blood vessels from mesenteric and cutaneous vascular beds of rodents were not dilated by ghrelin or non-peptide ghrelin receptor agonists at up to 100 times their EC50. Nevertheless, peripheral application of agonists lowered blood pressure and decreased splanchnic vasomotor nerve activity. In preliminary experiments (n = 3) vagotomy prevented these hypotensive and sympathoinhibitory effects. Ghrelin receptor antagonists were synthesised and characterised using cells tranfected with human or rat ghrelin receptors. Antagonists raised the blood pressures of anaesthetised rats by about 10 mmHg. Direct application of receptor agonists to the lower thoracic or lumbar spinal cord raised blood pressure. Ghrelin receptor agonists applied peripherally reduced firing in presympathetic vasomotor neurons of the rostral ventro-lateral medulla. **Conclusions:** Ghrelin receptors at different sites in vasomotor pathways affect blood pressure in different ways. Circulating ghrelin may reduce blood pressure by action on vagal afferent nerve endings.

POS-TUE-030

GHRELIN RECEPTOR EXPRESSION IN AUTONOMIC PREGANGLIONIC NEURONS

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Purpose: In situ hybridisation and functional studies reveal that subsets of autonomic preganglionic neurons express ghrelin receptors (GHSR). This study investigated which preganglionic neurons express GHSR. **Methods:** We used 20 mice expressing enhanced green fluorescent protein (EGFP) under control of the promoter for GHSR (characterised previously: Venables et al., Cell Tissue Research, 2011). Fast Blue was injected into target organs of another 4 reporter mice under anaesthesia. To retrieve tissues, mice were anaesthetised with a xylazine/ ketamine mixture and perfused with fixative or killed by exsanguination. Spinal cords and ganglia were fixed in formaldehyde or formaldehyde / picric acid. Cryostat sections were prepared for immunohistochemistry using anti-EGFP and antibodies to neuronal markers. **Results:** EGFP was detected in cell bodies of a proportion of autonomic preganglionic neurons from all levels, in varicose nerve terminals in all sympathetic chain, prevertebral and pelvic ganglia and in non-varicose fibres associated with ganglia. Spinal cord: EGFP-immunoreactive (IR) neurons were present in both the intermediolateral cell columns and central autonomic areas and overlapped with both NOS and CART. Sympathetic chain ganglia: most neurons were surrounded by EGFP-IR terminals. Stellate ganglion: EGFP-IR terminals surrounded most cells; few ChAT-IR neurons had surrounding EGFP-IR terminals. Superior cervical ganglion: EGFP-IR terminals innervated neuronal subgroups. NPY-IR neurons projecting to eye had terminals around them, but large NPY negative neurons projecting to submaxilliary gland were uninnervated. Celiaco-mesenteric ganglion: almost all neurons were surrounded by EGFP-IR terminals; VIP-IR terminals that project from the gut were EGFP negative. **Conclusions:** Ghrelin receptors are expressed by specific preganglionic neuron subgroups, including vasoconstrictor pathway neurons and neurons controlling gut function.

POS-TUE-031

EVIDENCE FROM BILATERAL RECORDINGS OF MUSCLE SYMPATHETIC NERVE ACTIVITY FOR LATERALIZATION OF VESTIBULAR CONTRIBUTIONS TO CARDIOVASCULAR CONTROL

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Purpose: Using low-frequency (0.08-0.18 Hz) sinusoidal galvanic vestibular stimulation we recently showed that two peaks of modulation of muscle sympathetic nerve activity (MSNA) occurred for each cycle of stimulation: a large peak associated with the positive peak of the sinusoid (defined as the primary peak) and a smaller peak (defined as the secondary peak) related to the negative peak of the sinusoid (Hammam et al., 2011). However, these recordings were only made from muscle fascicles of the left common peroneal nerve. To investigate potential lateralization of vestibulosympathetic reflexes, in the current investigation concurrent recordings were made from both the left and right common peroneal nerves. Methods: Tungsten microelectrodes were inserted into motor fascicles of the right and left common peroneal nerves in ten healthy individuals. Bipolar binaural sinusoidal GVS (±2 mA, 100 cycles) was applied to the mastoid processes at 0.08 Hz. Results: Cross-correlation analysis revealed that vestibular modulation of MSNA on the left side was expressed as a primary peak related to the positive phase of the sinusoid and a secondary peak related to the negative phase of the sinusoid. Conversely, on the right side the primary and secondary peaks - while still related to the positive and negative peaks – were reversed: the secondary peak on the right coincided with the primary peak on the left and vice versa. Conclusions: We believe the results support the conclusion that the left and right vestibular nuclei send an ipsilateral and contralateral projection to the left and right medullary output nucleus from which MSNA originates (RVLM). This causes a “flip flop” patterning between the two sympathetic outflows: when the burst is high on the left it is low on the right, and when the burst is low on the left it is high on the right. Hammam E, James C, Dawood T & Macefield VG (2011) Low-frequency sinusoidal galvanic stimulation of the left and right vestibular nerves reveals two peaks of modulation in muscle sympathetic nerve activity. Exp Brain Res 213: 507-514.

POS-TUE-032

DIFFERENTIAL SYMPATHETIC REFLEX RESPONSES TO MICROINJECTION OF NEUROMEDIN U IN RAT ROSTRAL VENTROLATERAL MEDULLA

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Purpose: The rostral ventrolateral medulla (RVLM) regulates sympathetic vasomotor outflow and forms an essential central component of sympathetic reflexes. Neuromedin U, a brain-gut peptide, increases sympathetic nerve activity (SNA), mean arterial pressure and heart rate after intracerebroventricular injection. However, the central areas that mediate these cardiovascular effects have not been clearly elucidated. Methods: In urethane-anaesthetized, vagotomised and artificially ventilated male Sprague-Dawley rats (n = 20) the effects of bilateral microinjection of NMU into RVLM on somatosympathetic, baro- and chemo- reflexes were investigated. Results: Microinjection of NMU (100 pmol) into RVLM caused biphasic responses, a brief hypertension and sympathoexcitation followed by prolonged hypotension and sympathoinhibition. NMU (100 pmol) attenuated both sympathoexcitatory peaks of splanchnic SNA (SSNA) (47 ± 8 %, $P<0.01$, and 43 ± 7 %, $P<0.001$, respectively, as compared to control (100%)) and lumbar SNA (LSNA) (54 ± 4 %, $P<0.001$, and 51 ± 12 %, $P<0.05$, respectively, as compared to control (100%)) of somatosympathetic reflex. NMU, at both excitatory and inhibitory phases, attenuated the pressor, tachycardia, sympathoexcitatory (both SSNA and LSNA) and phrenic nerve frequency responses to hypoxia in RVLM. The sympathoexcitatory (SSNA: 40 ± 5 %; LSNA: 49 ± 8 %; $P<0.01$) and pressor (33 ± 11 %, $P<0.01$) responses to hypercapnia are reduced while the tachycardia response (151 ± 14 %, $P<0.05$), is increased, as compared to control (100%), by NMU. Baroreflex sensitivity of SSNA is increased but that of LSNA is decreased following NMU injection. Conclusion: The present study provides functional evidence for a complex differential modulatory activity of NMU on the sympathetic reflex responses that are integrated in RVLM.

POS-TUE-033

SYMPATHOEXCITATION IN OBSTRUCTIVE SLEEP APNOEA ASSESSED BY CONCURRENT MICRONEUROGRAPHY AND FMRI

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Purpose: Using direct microelectrode recordings of muscle sympathetic nerve activity (MSNA) concurrently with functional magnetic resonance imaging (fMRI) of the human brain, we have been able to identify areas in the brain responsible for the generation of MSNA in healthy subjects. Here we aimed to identify functional changes in the brains of patients with Obstructive Sleep Apnoea (OSA), who are known to develop neurogenic hypertension. Methods: MSNA was recorded via a tungsten microelectrode inserted into the common peroneal nerve in 12 patients. Continuous blood pressure was recorded with MSNA in the laboratory prior to scanning. Gradient echo, echo planar BOLD fMRI was performed using a 3T scanner (Phillips, Achieva). Two hundred scans were collected continuously in a 4s-ON, 4s-OFF protocol (46 axial slices, TR = 8s, TE = 4s, flip angle = 90 deg, raw voxel size = 1.5x1.5x2.75 mm). Total sympathetic burst amplitudes were measured from the RMS-processed mean voltage amplitude during the 4s-OFF period. Results: All patients showed elevated levels of MSNA compared to healthy control subjects: 39±4 bursts/min and 43±6 bursts/100 heart beats. Mean blood pressure and heart rate were 138/79±6/2 mmHg and 75±4 bpm. Stable recordings of MSNA were maintained in all patients during fMRI, allowing us to correlate individual bursts with BOLD signal intensity; these data are currently being analysed. Conclusion: We have successfully extended our approach of concurrent microneurography and fMRI to a patient population, and hope to be able to identify changes in the brain that lead to the sympathoexcitation associated with OSA.

POS-TUE-034

AUTONOMIC MARKERS OF EMOTIONAL PROCESSING: SKIN SYMPATHETIC NERVE ACTIVITY IN HUMANS DURING EXPOSURE TO EMOTIONALLY-CHARGED IMAGES

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The sympathetic innervation of the skin primarily subserves thermoregulation, but the system has also been commandeered as a means of expressing emotions. While it is known that the level of skin sympathetic nerve activity (SSNA) is affected by anxiety, the majority of emotional studies have utilised the galvanic skin response (GSR) as a means of inferring increases in SSNA. Purpose: The purpose of this study was to characterise the changes in SSNA when showing subjects neutral or emotionally-charged images from the International Affective Picture System (IAPS). Methods: SSNA was recorded via tungsten microelectrodes inserted into cutaneous fascicles of the common peroneal nerve in 10 subjects. Neutral images, positively-charged images (erotica) or negatively-charged images (mutilation) were presented in blocks of 15 images of a specific type, each block lasting 2 minutes. Images of erotica or mutilation were presented in a quasi-random fashion, each block following a block of neutral images. Results: Both images of erotica or images of mutilation caused significant increases in SSNA, coupled with sweat release and cutaneous vasoconstriction, but the increases in SSNA were greater for mutilation. Interestingly, a previous study showed that viewing images of mutilation had no effect on muscle sympathetic nerve activity, further emphasising the differential control of sympathetic outflow to muscle and skin. Conclusion: We conclude that SSNA, comprising cutaneous vasoconstrictor and sudomotor activity, increases with both positively-charged and negatively-charged emotional images. Measurement of SSNA provides a more comprehensive assessment of sympathetic outflow to the skin than does the use of sweat release alone as a marker of emotional processing.

POS-TUE-035

PARASYMPATHETIC INPUT TO ENTERIC NEURONAL PATHWAYS IN THE DISTAL COLON OF THE GUINEA-PIG

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Sacral parasympathetic pathways play an important role in the control of distal colonic and rectal motility and defaecation. **Purpose**: we aimed to study the mechanisms and pathways by which sacral parasympathetic neurons affect distal gut motility in the guinea pig. **Methods**: Preparations of guinea-pig distal colon were removed from humanely killed guinea pigs and studied in vitro. Contractility was recorded with force transducers attached 15, 30, 45 and 60mm orad to the point where rectal nerves entered the gut. Electrical stimulation was applied to rectal nerves. In other preparations, biotinamide was applied in vitro to rectal nerves, then preparations were fixed and processed for immunohistochemistry. In some preparations, extracellular recordings from rectal nerves were combined with focal electrical stimulation to trace antidromically extrinsic pathways in the gut wall. **Results**: Antidromic tracing from single rectal nerve trunks revealed that single axons projected <20mm orally in the guinea pig rectum. Interestingly however, electrical stimulation of rectal nerves evoked contractions at all recording points up to 60mm orally, with an increasing latency at sites along the colon suggesting robust activation of ascending enteric pathways. Motor responses to rectal nerve stimulation were hexamethonium (400μM) sensitive. Confocal analysis of anterograde tracing showed a selective association of extrinsic axons with calretinin-immunoreactive (ascending) rather than nitric oxide synthase immunoreactive, descending enteric neurons (0.21±0.08 vs 0.14±0.08 appositions per unit surface area, 60 neurons, n=4, P<0.05). Calbindin-immunoreactive enteric neurons also received more extrinsic inputs than NOS-immunoreactive neurons (0.17±0.08 vs 0.09±0.08; 70 neurons, n=3, P<0.05). **Conclusion**: Sacral parasympathetic pathways modulate colorectal motility by selective activation of ascending enteric neuronal pathways, largely involving nicotinic cholinergic synapses.

POS-TUE-036

HEART FAILURE INCREASES BRAIN CYTOKINE EXPRESSION IN RATS

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Chronic heart failure (CHF) affects millions of people worldwide. The progression of the disease is accompanied by a multitude of neural-humoral changes including increases in sympathetic activity and increased circulating inflammatory cytokines. It has been suggested that circulating levels of inflammatory cytokines correlate well with disease severity. While circulating cytokines and their actions on the microvasculature, have been investigated, little is known about the cytokines produced within the brain - specifically in relation to disease states such as CHF. Using qPCR gene profiler arrays we screened for changes in 84 cytokine related genes within the brain of sham operated (control) and CHF rats. We identified significant (P<0.05, one-way ANOVA) changes of 2-fold or greater in 41 out of 84 genes. From this list we selected CCL2 and CX3CL1 for further study. CCL2 mRNA expression was increased 4.81-fold (P=0.0001) while CX3CL1 mRNA expression was increased 3.48-fold in CHF compared to sham rats (P=0.0004). Both these cytokines have previously been shown to play a significant role in modulating neuronal function. Immunohistochemistry revealed a robust and significant increase in the number of neurones expressing both CCL2 and CX3CL1, and their respective receptors, in the brain stem and hypothalamus of CHF rats. Double-immunofluorescence histochemistry revealed that while CX3CL1 is expressed both in neurones and glia, the receptor, CX3CR1, appears to be present only on neurones. These findings suggest that cytokines may play a role in modulating neuronal function and might be responsible for mediating some of the neural-humoral changes observed in CHF.

POS-TUE-037

PHARMACOLOGICAL ACTIVATION OF VISCERAL MECHANO-NOCICEPTORS IN THE GUINEA PIG ILEUM

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A major class of spinal mechanonociceptors to the gastrointestinal tract innervate intramural and extramural blood vessels, particularly arteries. They have mechanotransduction sites on vessels, often near branch points, and can be activated by mechanical distortion of their endings, for example by contraction and distension of the tissue. **Purpose**: We aimed to determine some of the pharmacological responses of intramural vascular afferents. **Methods**: The mucosa and muscularis externa were removed from a specimen of guinea pig ileum in vitro. Extracellular recordings and biotinamide dye fills were made of mesenteric nerve trunks and submucosal mechano-nociceptors were identified by responses to von Frey hairs applied to submucosal arteries and by responses to circumferential distension. **Results**: Capsaicin (1uM) added to the bath activated 13/14 mechano-nociceptor units tested. Noradrenaline (1μM) reliably activated 17/17 units tested (n=16). Responses to noradrenaline persisted in calcium-free Krebs solution, suggesting that they were direct, rather than being mediated via vascular or enteric smooth muscle. Concentration response curves revealed a threshold concentration near 0.1μM. Vascular afferents were activated by phenylephrine and isoprenaline suggesting involvement of both alpha and beta adrenoceptors. The muscarinic agonist, oxotremorine (1μM) also activated afferents, as did the nitric oxide donor, sodium nitroprusside (10μM). **Conclusion**: submucosal arteries receive innervation by sympathetic adrenergic and enteric cholinergic vasomotor neurons, in addition to containing transduction sites of spinal mechano-nociceptor nerve endings. The patterns of response identified in this study raise the possibility of close interactions between different functional classes of nerve endings in the submucosal perivascular plexus.

POS-TUE-038

BILATERAL AMYGDALA INJECTIONS OF ANTI-DBH SAPORIN CONJUGATE SUBSTANTIALLY IMPAIR SYMPATHETIC CUTANEOUS ALERTING VASOMOTOR RESPONSES (SCVARS)

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Background: Our laboratory has previously demonstrated that, in rats and rabbits, functional inactivation of the amygdala abolishes sympathetic cutaneous vasomotor alerting responses (SCVARs). We have now investigated whether immunotoxin- mediated destruction of noradrenergic innervation of the amygdala alters SCVARs. **Methods:** Under isoflurane anesthesia, Sprague Dawley rats (300-400 g) were implanted with ultrasound doppler flow probes (Iowa Inc, USA) around the tail artery and saporin conjugated to anti-DBH (100 ng/200nl, Advanced Targeting Systems) was injected bilaterally into the amygdala (AP 2.2 mm; ML 5.0 mm; DV 8.2mm). Tail probe cables were then passed subcutaneously and were connected to a headpiece fixed to the skull with dental cement. After one-week recovery unrestrained rats were placed in a quiet closed wooden box at 24-26°C. The tail artery blood flow signal (40 Hz sampling rate) was recorded, via a swivel device, with PowerLab (ADInstruments). Standardized alerting stimuli were administered and the effect on tail artery blood flow was analysed. **Results:** In control animals (Blessing et al., 2005), alerting stimuli reduced tail blood flow by 80±2 %. After bilateral immunotoxin injections alerting stimuli reduced tail blood flow by 19±1 % ($P<0.01$, n=6). Appropriate post-mortem immuno-histochemical analysis showed destruction noradrenaline containing nerve terminals in the amygdala surrounding temporal lobe and the medial prefrontal and insular cortices. Perikarya and the cell bodies in the locus coeruleus bilaterally destroyed, with variable damage to A1 and A2 noradrenaline neuron in the medulla oblongata. **Conclusions:** Destruction of noradrenergic amygdala and neighbouring forebrain regions substantially abolishes SCVARs. This suggests the noradrenergic innervation of the amygdala normally facilitates forebrain processes coupling perception of salients events to sympathetically mediated cutaneous vasoconstriction.

POS-TUE-039

DCC EXPRESSION ON THE MATURE ENTERIC NEURONS IN COLORECTAL CANCER AND CHEMOTHERAPY

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Purpose: The purpose of this study is to examine if colorectal cancer (CRC) and anti cancer chemotherapy have affected the netrin-1 receptor, DCC (deleted in colorectal cancer) of the enteric neurons. The investigation of DCC expression is imperative as it will guide us to see if netrin-1 can be used as a potential neuro-protectant for damaged enteric neurons induced by CRC and chemotherapy Methods: Murine CRC cells, CT26 (106 cells in 25μl of matrigel) were injected into a ceacum wall of the adult Balb/c mice. In a separate group, a chemotherapeutic drug, oxaliplatin (3mg/kg) was injected into mice intraperitoneally three times a week. The colon segments were taken from both CRC and chemotherapy groups at 3, 7 and 14 days (n=3 for each time point). Data were compared with sham-treated mice. Immunohistochemistry was performed on the colon segments revealing myenteric plexus, and DCC expression was analysed by confocal microscopy. Results: In sham-treated mice (n=3), almost all neurons in the myenteric ganglia expressed DCC receptors. The cell bodies of the enteric neurons were labeled with DCC but also a strong labeling of the nuclei of neurons was apparent. In tumor-bearing mice, metastases in the colon were observed by day 14, and the structure of myenteric ganglia was disrupted. In oxaliplatin-treated mice, it appears that DCC expression was found in some ganglia at day-7 and was absent in the most of ganglia at day 14. Conclusion: The expression of DCC on the enteric neurons was severely affected by chemotherapy. This implies that netrin-1 may be able to prevent the loss of DCC expression.

POS-TUE-040

AXONAL DAMAGE CAUSED BY COLORECTAL CANCER AND ANTI-CANCER CHEMOTHERAPY

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Purpose of this study was to investigate the morphological damage to the enteric neurons innervating gastrointestinal wall following the induction of cancer and anti-cancer chemotherapy. Methods: Oxaliplatin (3 mg/kg) was administered via i.p. injections three times a week for 3 weeks. The colorectal cancer model was established by injecting $1x10^6$ C26 cells into the ceacum wall of the Balb/c mice. Cross sections of the distal colon segments were examined histologically and quantified immunohistochemically in oxaliplatin and sham-injected mice at 3, 7, 14 and 21 days (n=3 per time point) following injections, and in colorectal cancer and sham-operated mice at 3, 7 and 14 days post-surgery. Nerve fibre bundles were labeled with anti-β Tubulin antibody followed by double-staining with antibodies specific to different types of enteric neurons. The number of nerve bundles was visualized and quantified in mid-villi sections. Results: This study provides evidence that significant axonal damage and axonal loss occur at 3-21 days after induction of colorectal cancer and anti-cancer chemotherapy in the mouse colon. Neurons projecting to the mucosa that were affected by colorectal cancer and anti-cancer chemotherapy include Dogiel type II neurons from the myenteric plexus which functionally are the intrinsic primary afferent neurons. Conclusion: This is the first study of the changes in enteric neurons following colorectal cancer and anti-cancer chemotherapy in the mouse colon. Colorectal cancer and anti-cancer chemotherapy cause damage to the neuronal processes in the mucosa which might underlie functional changes in the gut.

POS-TUE-041

EOSINOPHILS AND EOSINOPHIL-REGULATORY MOLECULES AS THERAPEUTIC TARGETS FOR THE TREATMENT OF INTESTINAL INFLAMMATION

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Purpose: Eosinophils have a crucial role in the pathophysiology of Inflammatory Bowel Diseases. Our previous results suggest that changes in the properties of the enteric neurons following induction of inflammation persist due to ongoing mucosal inflammation indicated by increased eosinophil numbers in the lamina propria after the inflammatory insult. Eosinophil accumulation is modulated by a subfamily of chemokines, termed eotaxins, specific chemoattractants for eosinophils. In the present study we will investigate the effects of eotaxin-1 inhibitors and its receptors, CCR3, in prevention of functional changes in the intestine after TNBS-induced inflammation. Methods: Inflammation was induced by injection of TNBS (30mg/kg in 30% ethanol) into the guinea-pig colon. Segments of the inflamed colon were collected 24 hours post-TNBS or sham treatment and compared to controls. One group of TNBS treated guinea-pigs were given CCR3 antagonist by intraperitoneal injection 1 hour prior to administration of TNBS to block eotaxin-1 receptor and hence block accumulation of eosinophils. Function of the colon was assessed by video imaging motility experiments. eosinophil presence was quantified by means of both histological and immunohistochemical analysis. Immunohistochemistry sections were double labelled with β-tubulin and anti-eosiophil-derived neurotoxin. This double labelling enabled visualisation of EDN location in respects to neuron processes. Histology sections were counterstained with haematoxylin and eosin and used to assess histological damage and quantify eosinophil presence. Results: Inflammation causes significant inhibition of colon motility. Block eotaxin-1 receptor significantly inhibited eosiophil-derived neurotoxin and number of eosinophils and improved colonic motility. Conclusion: Inhibitors of eotaxin-1 and its receptors alleviate functional changes in the intestine that occur following TNBS-induced inflammation.

POS-TUE-042

PREMOTOR SYMPATHETIC NEURONS ACTIVATED BY NOVELTY AND EXERCISE

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Purpose: It has been proposed that the vasopressor premotor sympathetic neurons of the rostral ventrolateral medulla (RVLM) represent a final common pathway for all sympathetically-mediated pressor responses, including those associated with stress. However, conditioned fear is not associated with RVLM or adrenergic C1 activation but with activation of presympathetic neurons in the perifornical and paraventricular hypothalamus (PeF, Pa) and in the A5 noradrenergic group. The aim was to identify the presympathetic groups of 2 other stressors also associated with pressor responses, novelty and forced exercise. **Methods**: The retrograde tracer Cholera toxin B (CTB) was injected bilaterally into the upper thoracic cord (T2-T3). Two weeks later the rats were exposed to a new environment (Novelty) or forced to exercise (treadmill, 10m/min) both for 30 min. Two hours later the rats were perfused and their brains analysed for double immunolabelling of Fos and either CTB or tyrosine hydroxylase (TH). **Results**: Compared to Rest (n=6), the percentage of CTB or TH labelled neurons double labelled with Fos after Novelty (n=6) and Exercise (n=5) was significantly greater ($p<0.05$) in PeF (22.4±1.7% and 23.0±0.9%, respectively vs 13.0±1.9%), Pa (17.9±1.3% and 17.1±1.7%, vs 6.6±1.9%) and A5 (CTB, 16.7±2.7% and 12.2±2.3 % vs 5.8±2.2%; TH, 31.4±7.4% and 15.9±1.6% vs 6.4±2.4%), but not raphe pallidus (20.4±3.2% and 23.5±3.8% vs 22.8±4.2%), raphe magnus (13.2±2.5% and 14.4±2.2% vs 15.6±3.1%), rostral ventromedial medulla (19.3±3.7% and 19.7±2.2% vs 21.3±2.2%), RVLM (15.4±2.9% and 12.6±2.1% vs 13.1±3.1%) or C1 (9.9±2.5% and 15.1±5.7% vs 8.4±1.9%).**Conclusion**: Fos labelling suggests that the pattern of premotor sympathetic activation associated with novelty and forced exercise is the same as with Fear, that is, no activation of RVLM/C1 but activation of Pa, PeF and A5.

POS-TUE-043

EXPRESSION OF ANGIOTENSIN TYPE 1A RECEPTORS IN C1 NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA IS REQUIRED FOR THE PRESSOR RESPONSE TO ANGIOTENSIN II

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Purpose: Angiotensin II (AngII) acts via its type 1A receptor ($AT_{1A}R$) in the rostral ventrolateral medulla (RVLM) to modulate blood pressure. Within the RVLM there are two principal cell types, catecholaminergic C1 neurons and non-C1 neurons. Whilst AngII is known to excite C1 neurons, it is not known whether this is sufficient for the pressor response *in vivo*. **Methods:** To examine which cells in RVLM express the $AT_{1A}R$ we used a transgenic mouse in which green fluorescent protein (GFP) expression is under the control of the $AT_{1A}R$ promoter. Mice (n=3) were anesthetized and perfused with 4% paraformaldehyde to enable double-labeling immunohistochemistry to detect GFP and tyrosine hydroxylase (TH). To determine whether $AT_{1A}R$ expression in C1 neurons is necessary for the pressor response to AngII in RVLM, we used a transgenic mouse with a conditional $AT_{1A}R$ allele ($AT_{1A}R^{fl/fl}$). Cre-recombinase (Cre) expression was induced by injecting a lentivirus into the RVLM of adult mice (n=6) in which transgene expression was under the control of a *phox2* binding site promoter. Control $AT_{1A}R^{fl/fl}$ mice (n=6) were injected with the same virus with a GFP transgene. Four weeks after viral injection the mice were anesthetized and instrumented for recording blood pressure. Ang II (50 nL of 1 mM) and glutamate (10 nL of 10 mM) were injected into the RVLM. Results: Double-labeling immunohistochemistry revealed that the majority of C1 neurons expressed the $AT_{1A}R$. A small number of $AT_{1A}R$-expressing non-C1 neurons were observed. Cre expression in the RVLM of $AT_{1A}R^{fl/fl}$ mice did not affect the response to microinjection of glutamate into the RVLM (Cre: 16±(SD)2.5 mmHg; GFP: 14±(SD)2.2 mmHg N.S.), but significantly attenuated the response to AngII (Cre: 3±(SD)3.4 mmHg; GFP: 17±(SD)5.2 mmHg $P \leq 0.001$). Conclusion: Expression of the $AT_{1A}R$ occurs endogenously in C1 neurons and is essential for the pressor response to AngII in the RVLM.

POS-TUE-044

EFFECTS OF ANTI-CANCER CHEMOTHERAPY ON GASTROINTESTINAL MOTILITY

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Anti-cancer chemotherapy has significant gastrointestinal side-effects. Diarrhoea, nausea and vomiting leading to malnutrition, dehydration, and rapid weight loss are due to the gastrointestinal damage. The traditional view is that gastrointestinal side-effects of anti-cancer drugs are due to mucosal damage. However, the physiological functions of the gastrointestinal tract such as motility, secretion and nutrient absorption are controlled by the enteric nervous system (ENS) innervating the intestine. Neurotoxic effects of anti-cancer drugs on the ENS have not been studied in depth and may contribute to the side-effects of these drugs. **Purpose:** This study investigates the effects of anti-cancer chemotherapeutic drug, oxaliplatin, on gastrointestinal motility. **Methods:** The dose-effect of oxaliplatin (1nM-10μM) on motility of the colon has been studied in organ-bath experiments in control Balb/c mice. In a separate group of experiments oxaliplatin (3 mg/kg) was administered via i.p. injections three times a week for 3 weeks. Segments were collected from oxaliplatin and sham-injected mice at 3, 7, 14 and 21 days (n=6 per time point) following injections. Peristaltic contractions were induced by increasing intraluminal pressure with physiological solution. Spatiotemporal maps were used to obtain quantitative measurements of the threshold for activation of propagating contractions, their frequencies, duration, speed, distance propagated and amplitudes. **Results:** Oxaliplatin inhibited the frequency of contractions with EC50 of 100nM. The frequency of contractions started recovering after 30min of washing out of oxaliplatin. In vivo oxaliplatin treatment caused inhibition of motility starting from the day 3 of treatment. **Conclusion:** Oxaliplatin inhibits neutrally-dependent activity in the colon.

POS-TUE-045

RENAL SYMPATHETIC NERVE ACTIVITY IN A RODENT MODEL OF CHRONIC KIDNEY DISEASE

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Chronic renal disease (CKD) is characterized by an marked increase in overall sympathetic nerve activity (SNA), assessed by both direct and indirect measures including muscle SNA and circulating catecholamines. Very few studies have directly assessed renal SNA in CKD. We have examined renal SNA and its baroreflex control in the Lewis polycystic kidney (LPK) rat. In urethane anaesthetised LPK (n=9) and Lewis control animals (n=11), baseline parameters of heart rate (HR), blood pressure, renal SNA and aortic depressor nerve activity (ADNA), and their responses to phenylephrine (10-50 µg/kg) and sodium nitroprusside (50-70 µg/kg) were determined. The MAP-nerve activity relationship was characterized using four-parameter sigmoid regression. At baseline, LPK rats had higher HR (351±7 vs 414±10 bpm), SBP (113±4 vs 174±8 mmHg) and renal SNA (3.5±0.5 vs 6.6±0.8 µV) (P≤0.05) however ADNA levels were not different (0.9±0.2 vs 1.0±0.3 µV). The RSNA baroreflex function curve was shifted to the right towards a higher MAP range, with a marked reduction in the gain (2.3±0.2 vs 1.1±0.2 %mmHg, P≤0.01) and magnitude of reflex sympathoinhibition. ADNA baroreflex function curves were shifted rightward, but there was no significant change in sensitivity to increases in MAP, supporting an intact sensory afferent pathway that is operational at higher resting MAP. These results indicate that renal SNA is increased in the LPK model of CKD, and has impaired baroreflex control. However, the sensory afferent component of the baroreflex arc is intact, suggesting that the impairment is due to either altered central and/or efferent mechanisms. This has significant clinical implication with regard to the contribution of increased SNA to CKD progression and overall increased risk of cardiovascular events.

POS-TUE-046

A COMPUTATIONAL MODEL OF INTRINSIC SENSORY NEURONS OF THE GUT

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Purpose: Intrinsic sensory neurons (ISNs) of the enteric nervous system (ENS) respond to physiological stimuli such as muscle contractions, mucosal distortion and chemical changes in the lumen. The resulting ISN activity influences enteric neural networks to regulate intestinal function. ISNs express a large number of voltage and calcium gated ion channels. However, it is unclear how interactions between the different ionic currents produce both normal and pathological behaviours. **Methods:** We constructed a detailed computer model of ISNs. The model includes voltage-gated sodium and potassium channels, an N-type calcium channel, a big conductance potassium (BK) channel, a calcium dependent non-specific cation channel (CaNSC), intermediate conductance potassium (IK) channel, hyperpolarisation activated cation (I_H) channels and internal calcium dynamics. **Results:** The model reproduced typical action potentials and after-potentials observed in these neurons. Intracellular recordings (n=8) were made to determine the amplitude, time to peak and half-duration of the late after-hyperpolarising potential (AHP) in response to 1, 3, and 5 current pulses (250 pA at 50Hz). The properties of the late AHP were reproduced in the model via interactions between the different currents: CaNSC and IK for the time to peak; IK, I_H and internal calcium dynamics for the half duration; IK and internal calcium dynamics for the amplitude. Also, interactions between BK, CaNSC and IK were able to reproduce the observation that action potentials were mainly generated on every second current pulse. **Conclusions:** This is the most detailed model of ISNs developed to date and has identified how interactions between specific channels influence the properties of the late AHP.

POS-TUE-047

MECHANOSENSITIVITY OF VISCEROFUGAL NEURONS IN GUINEA-PIG COLON

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Viscerofugal neurons can modulate gastrointestinal motility through peripheral reflex pathways involving prevertebral ganglia. Indirect studies of viscerofugal neuron populations suggest they might be directly mechanosensitive. Previously, we showed that identified single viscerofugal neurons can be recorded extracellularly from mesenteric nerve trunks of in vitro preparations of guinea–pig intestine. **Purpose:** To study characteristics of viscerofugal neuron mechanosensory responses. **Methods:** Flat-sheet preparations of guinea-pig distal colon with or without circular muscle removed were studied in vitro. Mesenteric nerve trunks were dissected and recorded with extracellular electrodes. Single units, DMPP-sensitive, but capsaicin-insensitive, were considered viscerofugal neurons since we have shown that these criteria correlated statistically with the presence of viscerofugal neurons. Von Frey hairs (100-200mg) were used to probe focal DMPP-responsive sites. Circumferential length and tension was modulated pharmacologically under isotonic or isometric conditions. **Results:** Eighteen units were identified in preparations without circular muscle (N=6) by focal DMPP (1mM) application. All units responded promptly to von Frey hair probing in Ca^{2+}-free Krebs. Thirteen units (N=6), identified in preparations with circular muscle, were stretched 1-4mm above resting length. All units increased firing rate upon circumferential distension in Ca^{2+}-free Krebs. Under near-isometric conditions, the L-type Ca^{2+} channel agonist BAY K8644 (1µM) increased circumferential tension to 250% of basal tension (2.1±1.0g vs 6.1±2.5g, p<0.001, N=6) while viscerofugal neuron firing rate was unchanged (2.0±2.3 vs 2.0±2.0Hz, NS, paired t-test). Under near-isotonic conditions, BAY K8644 (10µM) increased contractility-associated changes in circumferential length (1.3±0.8mm vs 4.2±1.2mm, p<0.001, t-test, N=6). Viscerofugal neurons preferentially fired at greater circumferential lengths (17 units, 2-4g counter-loads, p<0.001, ANOVA). **Conclusion:** All viscerofugal neurons we identified were directly mechanosensitive which respond potently as length, not tension receptors.

POS-TUE-048

DIFFERENTIAL CONTRIBUTION OF TRP CHANNELS TO ACUTE AND CHRONIC VISCERAL MECHANICAL HYPERSENSITIVITY

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Purpose: TNBS colitis induces acute and chronic mechanical hypersensitivity in splanchnic colonic afferents. We hypothesized members of the TRP channel family are involved. Methods: Using an in vitro mouse colon preparation, we determined the effect of various TRP agonists on afferent mechanosensitivity. Colitis was induced by TNBS 0.1mL (130µg/mL) after which mice were allowed to recover for 7 (acute) or 28 (recovery) days. Afferents were studied and compared in healthy, acute and recovery conditions in the respective TRP+/+ and -/- mice. Laser Capture Microdissection (LCM) of colonic sensory neurons and QPCR analysis compared channel expression in each state. Results: The TRPV1 agonist Capsaicin (3µM) activated 60% of healthy serosal afferents, but only 20% of recovery afferents. Correspondingly, QPCR analysis showed TRPV1 expression was significantly reduced in recovery colonic neurons (P<0.01), suggesting TRPV1 expression and function was actually decreased long after resolution of inflammation. The TRPV4 agonist 5,6-EET (10µM) caused mechanical hypersensitivity in healthy (P<0.05; n=12) and acute (P<0.01; n=7) afferents, but not in recovery (P>0.05; n=10). The proportion of afferents responding to 5,6-EET was also reduced after inflammation (46% healthy vs. 13% recovery), suggesting TRPV4 has a transient role in mechanical hypersensitivity. The TRPA1 agonist AITC (40µM) caused mechanical hypersensitivity in healthy (P<0.01; n=10), acute (P<0.001; n=16) and recovery (P<0.01; n=9) afferents. The specificity of these effects were confirmed in TRPV1, TRPA1 and TRPV4 -/- mice respectively (n=8-15). Two other TRP channels, TRPV2 and TRPV3 also contributed to altered mechanosensitivity, with the TRPV2 agonist, probenecid, and the TRPV3 agonist, ethyl-vanillin, having contrasting effects between inflammatory and recovery states (P<0.05; n=8-12). Conclusion: The expression and function of TRPV1 and TRPV4 are actually decreased long after resolution of TNBS-induced colitis. TRPA1 plays an important mechanosensory role in all conditions, whilst TRPV2 and TRPV3 have differential roles between inflammatory and recovery states.

POS-TUE-049

THE EXTRACELLULAR CALCIUM SENSING RECEPTOR MEDIATES LOCAL INHIBITORY REFLEXES EVOKED BY L-AMINO ACIDS IN GUINEA PIG JEJUNUM

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Purpose Application of the aromatic amino acids L-phenylalanine (L-Phe) or L-tryptophan (L-Tryp) to the intestinal mucosa excites a reflex that triggers inhibitory junction potentials in the circular muscle. This involves release of 5-HT and/or ATP from mucosal enteroendocrine (EE) cells, but how EE cells detect amino acids is unknown. This study tested whether the extracellular calcium sensing receptor (CaSR), which is sensitive to aromatic L-amino acids, is involved in sensing L-amino acids in guinea pig jejunal mucosa. **Methods** Circular muscle cells were impaled close to intact mucosa and inhibitory reflexes evoked by applying L- and D- amino acids mucosally. Involvement of the CaSR was examined using the CaSR antagonist NPS 2143 and agonist cinacalcet. **Results** L-Phe and L-Tryp evoked significantly larger IJPs than their respective D-isomers when tested at the same location (N=4 or 6). The non-aromatic amino acids L-leucine and L-lysine were much less effective at producing IJPs at sites where L-Phe evoked them consistently. NPS 2143 at 10 µM and 30 µM reversibly reduced L-Phe evoked IJPs by 50% (N=6, P<0.001) and 60% (N=4, P=0.005) respectively. NPS 2143 had no effect on electrically evoked IJPs. Cinacalcet (10 µM or 30 µM) evoked IJPs indistinguishable from those evoked by the L-amino acids. These were abolished by tetrodotoxin (1 µM, N=3) and significantly reduced by PPADS (10 µM, 60% reduction, N=4) and tropisetron (10 µM, 50% reduction, N=4). Together PPADS and tropisetron reduced the IJPs by 75%. **Conclusion** Local inhibitory reflexes evoked by aromatic L-amino acids involve activation of the CaSR. CaSRs probably act as sensory transducers for luminal amino acids in guinea-pig jejunum.

POS-TUE-050

CHOLERA TOXIN INCREASES EXCITABILITY OF MYENTERIC, BUT NOT SUBMUCOSAL, INTRINSIC SENSORY NEURONS IN GUINEA-PIG JEJUNUM

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Purpose: Neural pathways mediating cholera toxin (CT)-induced hypersecretion in the gut have not been fully characterised. We examined whether CT treatment changes the firing properties of myenteric and submucosal intrinsic sensory (AH) neurons to determine whether such changes might contribute to the mechanisms underlying neurogenic diarrhoea. **Methods:** Segments of guinea pig jejunum were incubated for 90 min with saline plus CT (12.5 µg/ml) or CT + drug in the lumen. After washing CT away, preparations of myenteric or submucosal plexus were dissected keeping circumferenterially adjacent mucosa intact. Submucosal and myenteric AH neurons were impaled within 2 mm of intact mucosa and myenteric neurons were impaled further away from mucosa and in preparations lacking mucosa entirely. The number of action potentials fired and the duration of firing during depolarizing current pulses (500 ms duration, 50 – 350 pA) were determined. **Results:** Thirty CT-treated myenteric AH neurons fired more action potentials over a longer duration during 500 ms depolarizations than control neurons (n=23). This applied wherever they were impaled. In contrast, firing of submucosal AH neurons was unaltered by CT. The addition of tetrodotoxin (1 µmol/L) to the luminal incubation solution, or a combination of the neurokinin 1 (NK1) antagonist SR140333 (100 nmol/L) and NK3 antagonist SR142801 (100 nmol/L), prevented the CT -induced hyperexcitability in myenteric AH neurons. **Conclusions:** CT makes myenteric, but not submucosal, AH neurons hyperexcitable. Unlike secretomotor neurons that are only hyperexcitable when close to intact mucosa, this response is observed in AH neurons regardless of proximity to, or presence of mucosa. The effect is neurally mediated and depends on NK1 and/or NK3 tachykinin receptors.

POS-TUE-051

UNDERSTANDING THE MECHANISMS WHICH UNCOUPLE SMOOTH MUSCLE CELLS OF THE GUINEA PIG ILEUM FOLLOWING DISSECTION

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We have previously shown that for 120 minutes after setting up preparations of guinea pig ileum in vitro, inhibitory junction potentials are significantly suppressed together with input resistance, dye coupling and response to exogenous ATP. After responses have recovered, gap junction blockers cause similar effects. **Purpose:** to determine which components of the setting up procedures cause the loss of gap junction coupling. **Method**: Intracellular recordings and dye fills were made from circular muscle cells of guinea pig ileum using microelectrodes containing 5% carboxyfluorescein. **Results**: After preparations had reached full responsiveness, transiently reducing temperature to 14°C did not significantly suppress responses. Cutting all 4 edges of a responsive preparation did cause partial uncoupling, reducing IJPs from -17.2± 0.7mV to -9.5±1.5mV (p<0.001, n=12) with recovery in <60 minutes. RMP significantly hyperpolarized and dye coupling significantly reduced. Stretching preparations to 130% (n=5) and 150% (n=4) of original length caused a small reduction in IJPs for 20+ minutes (-22.3±1.9 mV to -15.4±2.0 mV, 130% stretch p<0.05) but no measurable uncoupling. Hyperpolarisations evoked by exogenous ATP also decreased transiently (-18.1±2.4 mV to -11.9±0.7, 130% stretch p<0.05). When preparations were set up without dissection (ie: mucosa attached), the initial suppression was reduced in both amplitude and timecourse. Setting up preparations in calcium-free solution did not prevent the initial suppression of responses. Dissecting preparations in 3µM indomethacin (n=3) or 10µM ketotifen (n=4) did not block initial suppression. **Conclusion:** Cutting and removing the mucosa of preparations uncouples gap junctions but this is not dependent on extracellular calcium. Mast cells, prostaglandin production and changes in temperature did not contribute to loss of coupling.

POS-TUE-052

ABLATION OF NEURONAL ANGIOTENSIN TYPE 1A RECEPTORS REDUCES BLOOD PRESSURE IN MICE

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Purpose: Basal blood pressure is tightly regulated and its dysregulation leads to hypertension. Currently, it is poorly understood how basal blood pressure tone is determined. Angiotensin II is a potent hormone that is important in cardiovascular function, thirst and fluid homeostasis. Its main effects are mediated through its type 1A receptor ($AT_{1A}R$) found in the brain, adrenals, kidney, heart and vasculature. Global deletion of the $AT_{1A}R$ significantly reduces basal blood pressure with part of this effect due to loss of the receptor from the kidney. We propose that loss of the $AT_{1A}R$ from neurons also contributes to this decrease in basal blood pressure. **Methods:** To test this, neuron-specific ablation of the $AT_{1A}R$ was induced using a Cre-Lox genetic approach. Mice with a conditional $AT_{1A}R$ allele were crossed with mice expressing CRE under the control of a neuron-enhanced nestin (neurofilament) promoter. Blood pressure was measured in conscious mice using a non-invasive tail cuff system. NestinCRE+ $AT_{1A}R$ fl/fl (n=8) and their NestinCRE- $AT_{1A}R$ fl/fl littermates (n=6) underwent 4-5 consecutive days of acclimatisation to the equipment per week, at the same time each day, for 4 weeks. After this period blood pressure was recorded, using the same protocol for 4 weeks. **Results:** NestinCRE+ $AT_{1A}R$ fl/fl animals have a significant reduction in systolic blood pressure compared to their littermates (NestinCRE+: 110mmHg±(SD)4.7 vs NestinCRE-: 123±(SD)6.8mmHg; P=0.011). **Conclusion:** Neuronal $AT_{1A}R$ expression plays a role in determining basal blood pressure.

POS-TUE-053

BEES SELDOM GO BANG! MID-AIR COLLISION AVOIDANCE IN FLYING INSECTS

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Purpose: While considerable attention has been devoted to understanding how flying insects pursue and intercept other insects, relatively little is known about whether, and if so how flying insects avoid mid-air collisions with each other. We are investigating this question, which is important not only in the context of neurobiology but also in the design of aircraft vision systems for automated collision avoidance. **Methods**: Honeybees were trained to fly to and from a feeder placed at the far end of a narrow tunnel, and their flight trajectories were filmed, digitized and reconstructed in 3-D using two video cameras. The small cross section of the tunnel increased the likelihood of head-on collisions, thus enabling investigation of whether bees actively avoid collisions in high-density air spaces. **Results**: We observed a collision rate of 4.6%, filmed during 1500 head-on encounters (in over 7 hours of footage). This rate is significantly lower than that expected from random chance (17.9%), if the bees were flying 'blind' without attempting to avoid collisions. Detailed analysis of 90 encounters reveals that approaching bees begin to reduce their flight speed when they are separated axially by about 60 mm, and begin to veer away from each other at about the same axial separation. The transverse separation of the bees reaches a maximum value at an axial separation of about 20 mm prior to the point of crossing. **Conclusion**: Flying bees indeed actively avoid mid-air collisions with each other. Work is currently in progress to unravel the underlying strategies and dynamics.

POS-TUE-054

PROPERTIES OF THE NAVIGATIONAL PROCESS IN HOMING PIGEONS

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Purpose/Methods: In a novel approach, using the so called method of time lag embedding, we analyzed the tracks (n=164) of pigeons recorded with the help of miniaturized GPS recorders. We calculated two variables, the largest Lyapunov exponent to determine the predictability of the underlying process and the correlation dimension to estimate the number of factors involved. **Results:** A low Lyapunov exponent around 0.02, which remained rather constant over all calculations, indicates that the navigational process is almost deterministic. The distribution of the correlation dimension estimates, however, was significantly different from random, with distinctive peaks, at 3.3, 3.7 and 4.2, indicating that at least four independent factors are involved in the underlying process. Additional factors, as indicated by an increase in the correlation dimension, seem to be included as the pigeons approach their home loft. **Conclusion:** These findings suggest that the underlying cognitive process is highly flexible allowing individual navigational factors to be included as required and weighted independently. Neither the correlation dimension nor the Lyapunov exponent are affected by increasing familiarity of the pigeons with the terrain. This suggests that the same process controls flight across familiar as well as unfamiliar terrain.

POS-TUE-055

SLEEP THERAPY FOR FLIES

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Purpose: Sleep is common to most animals, yet its function remains mysterious. Here, we investigate a role for sleep in establishing attention processes, using the genetic model *Drosophila*. We are using the fly model to design sleep therapy strategies that might be used to correct cognitive disorders. **Methods:** Sleep is a state of decreased behavioural responsiveness; therefore one method to study sleep is to measure arousal thresholds. Arousal was probed in flies using mechanical stimuli over a 24-hour period. To manipulate sleep we used two strategies: sleep deprivation and sleep induction. We induced sleep in flies using the GABA agonist Gaboxadol, or by genetic activation of neurons in their central brain; sleep deprivation was mechanically induced. Animals with altered sleep regimes were tested for attention-related phenotypes by measuring visual responsiveness in a behavioural paradigm. **Results:** Mutations in the *dunce* gene affect cAMP signaling and cause learning and attention defects. Visual responsiveness in *dunce* animals is significantly greater than controls (20 experiments, $p<0.0001$), similar to sleep-deprived wild-type flies (28 experiments, $p<0.05$). Together, these data suggest that *dunce* attention defects may result from poor sleep. Indeed, we found that the, *dunce*[1] mutant has abnormal sleep patterns, as they were less aroused by day and more active at night, suggesting that *dunce* has interrupted sleep (n>34 flies, $p<0.05$). **Conclusion:** The learning mutant *dunce*[1] has reduced sleep and abnormal arousal levels, which may underlie its attention deficits. We propose that sleep induction will restore normal behaviour in *dunce* and other mutants with learning and attention defects.

POS-TUE-056

PRECISION LANDING OF FREELY FLYING BUDGERIGARS *(MELOPSITTACUS UNDULATUS)* ON A MOVING PERCH

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Purpose: We investigated how budgerigars approach and land on a moving perch. **Methods**: Budgerigars (n=4, Trials-40) were trained to take off from a hand-held perch and to land on a mechanically controlled, moving perch in a flight tunnel. The perch was executing simple harmonic motion. **Results**: The budgerigars predominantly timed their landing in such a way that the perch was at the point of maximum displacement (or minimum velocity), and hence was stationary in space at the time of touch down. However, on the rare occasions when birds landed on the perch when it was moving, the touchdown almost invariably occurred when the perch was moving towards the bird, rather than away from it. The birds then flapped their wings vigorously to decelerate, and hence reduce their speed relative to the perch, before landing on it. **Conclusions**: Our observations, which are the first to investigate the ability of birds to land on moving targets, show that this behaviour is a precisely controlled and timed event – one that has probably been honed by an evolutionary need to land safely on branches swinging in the wind.

POS-TUE-057

DO SIZE, BRIGHTNESS OR COLOUR MATTER? TARGET PREFERENCES IN AGGRESSIVE HONEYBEES

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Purpose: While much attention has been devoted to understanding vision, flight and navigation in foraging honeybees, we know relatively little about visually mediated behavior in aggressive bees. Here we investigate some of the visual attributes that an object must have in order to attract the greatest and the least attention in aggravated honeybees. **Methods:** We developed a mechanical method to 'aggravate' honeybees and investigated the intensity of their aggression (number of hits) towards different targets. Targets were presented as discs on a 46-inch TV screen and responses were filmed with a HD video camera (1080/50p) for detailed analysis. **Results:** (i) Analysis of 692 attacks to targets of different sizes reveals that aggression is highest when the target subtends an angle of about 5.7 degrees as viewed from the hive entrance. (ii) A systematic analysis of over 5000 attacks directed toward targets of various intensities and colours (grey, green, blue, or red), presented against backgrounds of various intensities and colours reveals that attack rates are highest when the target is dark compared to the background (i.e. presents a large negative contrast close to -1.0), and low when the target is brighter than the background (i.e. presents a positive contrast). To a first approximation, this is true irrespective of the colour of the target or the background (grey, green, blue, or red). **Conclusion:** Aggressive bees attack targets at high rates when the target is very dark relative to the background. Work is currently under way to unravel the detailed chromatic properties of target detection by aggressive honeybees.

POS-TUE-058

VISUAL FIXATION AND LEARNING IN TETHERED, WALKING HONEYBEES

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Purpose: The honeybee is an excellent model organism for studying behavioural responses to visual stimuli. Here, we designed a closed-loop walking paradigm for the bee, in order to study the interaction between visual attention and sensorimotor learning. **Methods:** Tethered bees walked on an air-suspended ball surrounded by a light emitting diode (LED) array displaying simple visual stimuli, such as a vertical green stripe. In closed loop, the angular position of the stripe was controlled by the bee's rotation of the ball. This allowed the bees to place the stimulus in front, which we term fixation. To measure the bee's ongoing responsiveness to the stimulus, random perturbations altered the stimulus location. Sensorimotor learning was investigated by altering the closed-loop gain between the bees' movements and the stripe location. **Results:** The bees showed preference for brightly lit vertical green bars compared to other objects of different shape, luminosity, or colour. Fixation on green bars was significant compared to unlit controls ($n=13$, $p<0.05$). During simultaneous presentation of a blue and green bar, the bee fixated significantly more on the green bar ($n=13$, $p<0.05$). When the gain between the bees' movements and the stripe location was altered, the bees could re-learn the relationship between its movements and that of the stimulus. This was shown with significantly improved fixation over time ($n=14$, $p<0.05$). Additionally, over time the bees improved fixation after a perturbation ($n=34$, $p=0.0015$), further indicating learning. **Conclusion:** Our walking bee paradigm shows that visual fixation improves over time, indicating it is a potentially a robust assay for attention and learning studies and possibly amenable to electrophysiology recordings. This would allow the neural correlates of cognitive functions such as attention, sensorimotor learning, and visual rivalry to be studied.

POS-TUE-059

COMPUTERISED COGNITIVE TRAINING IMPROVES BOOKKEEPING PERFORMANCE: A MATCHED-SAMPLING ACTIVE-CONTROL TRIAL

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Purpose: To assess the extent to which Computerised Cognitive Training (CCT) can improve job performance in healthy adults. **Methods:** A cohort of cognitively-intact business students (n=48, 71% female, mean age 21.4 ± 2 years) were assigned to either (a) 20 hours of supervised training on a commercially available CCT program, or (b) 20 hours of supervised computerised arithmetic training (active control) by a matched sampling procedure. Transfer of skills to performance on a 60-minute paper-based bookkeeping task was measured at three timepoints – baseline, after 10 hours and after 20 hours of training. **Results:** Repeated measures ANOVA found a significant Group X Time effect on productivity ($F=7.033$, $df=1.745$; 73.273, $p=0.003$) with a significant interaction at both the 10-hour (Cohen's effect size = 0.38, $p=0.014$) and 20-hour time points (Cohen's effect size = 0.40, $p=0.003$). Dichotomisation based on baseline productivity revealed a significant double interaction (Time X Group X Productivity), such that relatively more competent subjects at baseline showed the highest level of improvement ($F=3.090$, $df=6$; 80, $p=0.009$). No significant effects were found on accuracy. **Conclusions:** Here we report for the first time that CCT can enhance productivity in healthy younger adults on bookkeeping tasks with high relevance to real-world job performance. Against expectations, we found that more skilled individuals benefited more from training than less skilled individuals, suggesting that work performance can be enhanced even at higher skill levels. Generalisation of these results to the wider work environment will require further study.

POS-TUE-060

CORTICAL INPUT TO POSTERIOR PARIETAL AREA PE IN THE MACAQUE MONKEY

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Purpose: Modern histological methods have allowed the identification of many areas within the limits of Brodmann's area 5. Here we investigated the cortico-cortical afferent projections of one of these subdivisions, area PE, which extends from the caudal postcentral gyrus to the shoulder of the intraparietal sulcus. **Methods:** Macaque monkeys were anaesthetised with sodium thiopental, and received injections of fluorescent tracers (n= 6). **Results:** We found that PE forms specific connections with somatic, vestibular, and motor fields; however, the presence and strength of connections depends on the location of the injection sites. PE receives the strongest afferents from somatosensory area 2 and from the primary motor cortex. Together, neurons in these areas account for approximately 40% of total afferents. Additional input originates from other postcentral somatosensory fields, and from caudal parietal area PEc. The medial bank of the intraparietal sulcus contains substantial labelling only when injections involve lateral parts of PE. In the inferior parietal lobule, connections are observed mainly with parietal opercular areas PGop and PFop, and with the somato-vestibular retroinsular field. On the mesial brain, significant inputs arise from posterior cingulate areas (PEci and 23), and, to a lesser degree, from motor area 24. A final set of connections originates in the premotor cortex, in particular the supplementary motor area and the caudal subdivision of the dorsal premotor cortex. Compared to neighbouring posterior parietal areas, PE does not receive direct projections from visual extrastriate areas. **Conclusion:** Overall, the sensory and motor afferents to PE are consistent with the functions ascribed to this area in posture maintenance and goal-directed movement.

POS-TUE-061

LUMINANCE CODING IN MOUSE BINOCULAR VISUAL CORTEX

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Purpose: The aim of this study was to determine how luminance information received by the eyes is processed in primary visual cortex. **Method:** *In vivo* whole-cell current-clamp recordings were made from layer 5 and layer 2/3 pyramidal neurons in the binocular region of primary visual cortex in anesthetized mice. Luminance transients were presented to the contralateral eye, ipsilateral eye, or to both eyes together. Full field stimuli were delivered with light emitting diodes (LEDs). Stimulation of binocular visual space was achieved using a CRT monitor. In the latter case, mechanical eye shutters were used to restrict the visual stimuli to the ipsilateral or contralateral eye. **Results:** In experiments with LED illumination, changes in luminance evoked excitatory postsynaptic potentials (EPSPs) which were larger in amplitude, earlier in onset, and briefer in half width during stimulation of the contralateral (n=18) compared to the ipsilateral eye (n=11). Increasing luminance was associated with a progressive increase in EPSP amplitude, reaching saturation at high light intensities. Comparison of EPSPs evoked by stimulation of both eyes with the expected linear sum of responses generated following activation of each eye on its own indicated that the inputs were integrated supra-linearly during small changes in light intensity (linearity index: 1.3±0.3; n=8), but sub-linearly during large changes in light intensity (linearity index: 0.5±0.2; n=8). Similar results were obtained during luminance changes presented to only the binocular field of view using a CRT monitor. **Conclusion:** These data indicate that binocular luminance information is coded supralinearly at low intensities and sublinearly at high intensities in primary visual cortex, suggesting a cortical mechanism for gain control of light intensity.

POS-TUE-062

THE DIFFERENTIAL DISTRIBUTION OF BIPOLAR CELL TYPES IN THE PIGEON RETINA

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Purpose: In the pigeon retina, 8 types of bipolar cells have been previously identified; however, only bipolar cells located in the red field were classified (Mariani 1987). The aim of the present study is to classify bipolar cells throughout the pigeon retina and to determine whether there is a differential distribution of these cell types in areas of high acuity. **Methods:** Trephine punches (5mm) were taken from various retinal regions and cross-sectioned (n=16 pigeons). Bipolar cells (n = 313) were labelled using Diolistics, reconstructed with a confocal microscope, and classified according to Mariani (1987). **Results:** All of the bipolar cells described by Mariani were identified (B1-B8) and four additional groups with distinct dendritic branching patterns were defined (B9-12). The most prevalent bipolar cell type in the retina was B3 (17%) and the least prevalent was the B12 (0.6%) bipolar cell. All twelve types were present in the yellow field with B6 (21%), B4 (18%) and B3 (14%) bipolar cells being the most prevalent and B1 (1%) and B10 (1%) being the least prevalent. In the red field, eleven types (B1-B11) were identified; the B12 was not identified in this region. The most prevalent types in the red field were the B3 (20%), B10 (15%) and B8 (14%) bipolar cells. In the fovea, only five types (B2, B5, B6, B7, B10) were present with the B7 bipolar cell being the most prevalent (55%). **Conclusions:** There is a differential distribution of bipolar cell types in areas of high acuity. This suggests that high acuity information is conveyed through specialized pathways in the pigeon retina.

POS-TUE-063

STRAIGHTENING OUT THE MOUSE NEOCORTEX

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Purpose: With the ascendance of genetic manipulation techniques, the mouse is becoming a model for understanding the function and diseases of the human cerebral cortex. Thorough knowledge of mouse cortical anatomy, including homology to human neocortex, is of obvious importance for the validity of such research. **Methods:** 3D renderings of stereotaxic atlas sections were used as a starting point for a new map of functional regions in the mouse neocortex. Region boundaries were redrawn using a range of data from studies of hodology, physiology, gene expression and magnetic resonance imaging. This new map was dimensionally corrected to the stereotaxic atlas set and rendered in geometrically accurate views, delineating the cortical surface with unprecedented clarity and accuracy. **Results:** A new map of the mouse cortical surface, is presented in a dimensionally accurate rendering. In turn this map is used to back-project to stereotaxic sections to improve the placement of cortical region boundaries. **Conclusion:** Although regional divisions of function have long been acknowledged in the mouse neocortex, recent efforts to reconcile two dimensional stereotaxic and chemoarchitectonic anatomy with a three dimensional in vivo configuration have highlighted some difficulties with current atlases, and offer an opportunity for consolidating the anatomical foundations of research on the mouse cortex. It is hoped that the resultant maps will improve the basis on which neocortical research rests.

POS-TUE-064

THE AREAL DEVELOPMENT OF THE VISUAL CORTEX IS DEPENDANT ON EPHRIN-A2 AND EPHRIN-A5 SIGNALLING

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Purpose: Molecular guidance cues such as the ephrin ligands play a crucial role in the spatiotemporal organisation of the visual system. Whilst we have learnt that members of the ephrin-A family play a pivotal role in forming normal highly organised retinothalamic projections, limited work has been performed to demonstrate the impact the ephrin-As have in the formation of the areas of the visual cortex. Therefore, we sought to explore the organisation of the adult visual cortex in the mouse following the knockout of the ephrin-A2 and ephrin-A5 genes. **Methods:** Adult WT, ephrin-A2 $^{-/-}$, ephrin-A5$^{-/-}$ and ephrin-A2/A5$^{-/-}$ were used in this study (n=4 per genotype). Animals were deeply anaesthetised with sodium pentobarbitone and subsequently perfused with 4% PFA. We analysed areas V1, V2L and V2M with the mature neuronal marker, NeuN; activity marker, cFos; maturation marker,non-phosphorylated neurofilament marker (NNF); and, interneuronal markers,calbindin-D28K and parvalbumin. **Results:** A reduction in NeuN and cFos within layers II, III and IV of V1 in A2 (20%), A5 (25%) and A2/A5 (33%) knockouts was observed. All KO strains also exhibited a lack of definition in the boundaries, which delineate the V1/V2M and V1/V2L border as well as an absence of organisation of the six cortical layers, with the A2/A5 $^{-/-}$ being the most severely affected. **Conclusion:** Through the use of various immunohistochemical markers, we identified that both ephrin-A2 and/or ephrin-A5 influence not only the guidance of neurones but the lamination and the arealisation of the mouse visual cortex.

POS-TUE-065

SPIKE WAVEFORM ANALYSIS CAN RELIABLY IDENTIFY SOME RETINAL GANGLION CELL TYPES

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Purpose: Our perception of the visual scene is the result of massive parallel processing which begins at the very first retinal synapse. The information processing which is carried out by the retina is sent via the axons of 15-20 different retinal ganglion cell (RGC) types to a similar number of more central brain nuclei. Ultimately, the information sent down these axons is limited by the intrinsic physiological properties of each RGC type. Previously we have demonstrated that the overall intrinsic properties of RGC types vary quite dramatically. We have now undertaken a careful analysis of the spike waveforms generated by rat and cat RGC types to determine if they can be used to classify particular types, independent of their morphology. Methods: Rat and cat retinal wholemounts were prepared for in vitro recording. Using whole cell patch clamp techniques we have recorded the spike waveforms generated by each of the individual cell types (n = 279) and quantified many of their components using custom Labview software. Results: Using these data we have determined that individual cell types can be identified on the basis of their characteristic spike waveform alone. In particular, our data demonstrate that the rat A2 and its cat homologue, the alpha cell, can be unambiguously identified from other RGC types. Conclusions: Our data further support the idea that the intrinsic physiological properties of each individual ganglion cell type are unique.

POS-TUE-066

DOES SPATIAL ATTENTION AFFECT POST-SACCADIC ENHANCEMENT OF OCULAR FOLLOWING?

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Purpose: Activity of motion-sensitive cortical neurons is modulated by spatial attention and saccadic eye movements. Saccades also modulate the speed of reflexive tracking eye movements, termed the ocular following reflex (OFR). We investigated how spatial attention modulates post-saccadic enhancement of the OFR. **Methods**: Three subjects performed an OFR task with eight conditions. Subjects performed OFR with post-saccadic latencies of either 50ms (enhancement) or 350ms (non-enhancement) while maintaining covert spatial attention to one of three locations (the saccade target, away from the saccade target, or at fixation) or not attending. Subjects performed a working memory task at the attention location, requiring them to monitor a sequence of coloured rings presented at the cued location and indicate at the end of the trial whether a cued colour had been presented. Eye speed was averaged from 180 trials of each stimulus condition. **Results**: When attention was directed away from the saccade target, subjects had an increased number of false detections in the working memory task, demonstrating that task performance depends on the locus of spatial attention. Eye speed during OFR was faster for the enhancement condition than for the non-enhancement condition for all attention conditions but was not modulated by the presence or location of the attention stimulus. **Conclusions**: Our results suggest that spatial attention does not modulate post-saccadic enhancement of the ocular following reflex.

POS-TUE-067

POPULATION DECODING ALGORITHMS FOR CHANGE DETECTION AND DISCRIMINATION

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Purpose: Over what timescales is neuronal activity informative about constant, and changing, stimuli? To explore this issue, we recorded from MT and MST neurons in two macaque monkeys performing a task requiring the detection and discrimination of unpredictable speed changes. Previously, we have reported that on timescales of tens of milliseconds, the activity of single neurons encodes the sign of the speed change and can be decoded to predict the animals' behavioral judgments. **Method:** Here, we examine maximum-likelihood methods of decoding the responses of a population of neurons in order to predict the sign and timing of speed changes. We focus on how the raw spike trains of the population might be filtered, or pre-processed, to facilitate change detection and discrimination on behaviorally relevant timescales. **Results:** The most successful decoder, which had similar performance to the animal's behavior, was designed to simultaneously categorize recent speed variations using two independent change detectors: faster versus not faster, and slower versus not slower. We were unable to decode veridical speed throughout a trial. The decoder only performed better than chance if the raw spiking times were pre-processed to give a spike rate derivative. This derivative was formed by taking the difference between spike trains convolved with exponential functions with short and long time constants. The optimal prefilter time constants were 20 ms and 120 ms, with these times critically affecting the nature of the decoder outputs, including discrimination performance, the rate of false detections (noise susceptibility), and the timing of when changes were correctly detected. **Conclusion:** Stimulus changes, but not veridical stimulus properties, can be reliably decoded from short (~100 ms) timescales of activity across a neuronal population.

POS-TUE-068

670NM RED LIGHT: PROTECTION AGAINST RETINOPATHY OF PREMATURITY AND LOW DEVELOPMENTAL WEIGHT

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Purpose: Retinopathy of Prematurity (ROP) is a vasoproliferative disorder that can lead to blindness. The incidence and severity of ROP correlates with the extent of prematurity (<32 weeks) and low birth weight (<1.2kg). Red light irradiation at 670nm wavelength increases cellular differentiation, proliferation and wound repair. In the retina it protects photoreceptors from the effects of a number of noxious stimuli including toxins, bright white light exposure and oxygen-induced degeneration. We used an established mouse model for ROP (Oxygen Induced Retinopathy - OIR model) to determine the effects of 670nm light on vasculature development. **Methods:** Experimental C567/Bl6j pups were exposed to 75% oxygen from p7-12, then normoxia p12-p17 (OIR); received 9 J/cm2 of 670nm light daily from p7-17 (670nm); or both OIR and 670nm treatment. Normal mouse pups received no treatments. 'OIR' and 'OIR+670nm' animals were sacrificed at P17, the retinas fixed, isolated and prepared as wholemounts. Retinal vasculature was labelled using Griffonia simplicifolia lectin (Sigma L2895), and quantitatively analysed for changes in vascularisation patterns including neovascularization, vaso-obliteration and peripheral vessel branching. To assess the effects of 670nm light on development, a group of normal and '670nm-only' animals were weighed and measured daily. At P21 their organs were harvested, weighed and inspected for abnormalities.**Results:** Animals treated with 670nm (only) were significantly heavier than untreated normal mouse pups. No histological abnormalities were observed in organs including eyes, retina, kidneys, liver, spleen, heart, thymus, lungs and brain, although brain and lung weight were higher in the 670 nm light treated group. Compared to OIR animals, OIR + 670nm animals had significantly lower levels of both neovascularisation, vaso-obliteration and unique peripheral branching pattern. **Conclusion:** Exposure to 670nm light may provide a novel preventative strategy against ROP and problems associated with low birth weight.

POS-TUE-069

SINUSOIDAL STIMULATION OF RETINAL GANGLION CELLS: COMPUTATIONAL MODEL AND EXPERIMENTAL RESULTS

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Purpose: Current technology to restore visual sensitivity in blind patients is entirely non-selective about which retinal ganglion cells (RGCs) are activated. The axons of RGCs make up 15-20 parallel pathways, each of which encodes different aspects of the visual scene. Non-selective stimulation, therefore, can lead to nonsense stimulation. For example, activating both ON and OFF RGCs in the same retinal location is perceptually impossible. We have therefore sought to determine a means to activate either ON or OFF RGCs selectively. Methods: Whole-cell current clamp recordings from 39 RGCs were obtained by standard procedures and classified anatomically into ON (3), OFF (3) or ON-OFF (7) types. RGCs were stimulated with sinusoidal currents of varying frequency, 1-60 Hz. Numerical simulations of single-compartment Hodgkin-Huxley-type neurons were carried out in NEURON. The models of ON and OFF cells were constrained based on their published intrinsic electrophysiology and compared with experimental data. Results: Responses of ON and OFF RGCs to sinusoidal current injections differed substantially. Where both ON and OFF RGC types responded with bandpass characterstics peaking at 5-10 Hz, OFF cells were able to continue spiking at frequencies up to 60 Hz while ON cells failed to respond at frequencies above 20 Hz. 11 unidentified cells showed responses similar to OFF cells, 6 unidentified cells showed responses similar to ON cells. Conclusions: This study suggests that it may be possible to preferentially stimulate OFF cells over ON cells using sinusoidal stimulation of frequencies above 20 Hz.

POS-TUE-070

EFFECTS OF CONTRAST ADAPTATION ON THE CLASSIC RECEPTIVE FIELD OF OFF-ALPHA RETINAL GANGLION CELLS OF THE MOUSE

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In the retina, ganglion cells (RGCs) adapt to contrast. The reduction of RGC response during exposure to high contrast arises from two main mechanisms: reduction of glutamate release from bipolar cells, and recruitment of postsynaptic mechanisms. RGCs receive inhibitory as well as excitatory inputs, so a reduction in excitatory input may change their relative weights and lead to a change in the spatial profile of the receptive field. **Purpose:** To determine how contrast adaptation impacts on the receptive field properties of Off-Alpha RGCs, we investigated the spatial profile of excitatory and inhibitory inputs before and after prolonged exposure to high contrast. **Methods:** Recordings were made in loose patch, for spike recordings, and whole-cell configuration for conductance analysis, in whole-mount retinas of mouse. **Results:** Spike response during contrast adaptation showed significant reduction in amplitude but no change in the spatial profile of the receptive field (n=9). Conductance analysis of responses to preferred-contrast stimulus (n=8) showed significant reduction in amplitude and sharpening of the area response for the excitatory inputs but not for disinhibition. Inhibition recruited during light increments was strongly reduced in amplitude and more tuned during adaptation. **Conclusions:** Adaptation led to no changes in disinhibition (which is a major driving force of response in these cells), so the impact of adaptation on spiking response was less than expected from changes observed in excitatory inputs. Since inhibition recruited during light increments was reduced by adaptation but disinhibition during light decrement was not, this may suggest that these two sources of inhibition to Off-alpha cells involve distinct synaptic mechanisms.

POS-TUE-071

THE DYNAMIC REPRESENTATION OF EYE POSITION IN PRIMARY VISUAL CORTEX

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Visual input would be of little use if not accompanied by knowledge of eye position; indeed, it is the combination of these signals that allows the brain to localise and interact with objects meaningfully. Although eye-position signals have been observed throughout visual cortex – including the primary visual area (V1) – little is known about how well such signals represent the eye during fixation and across eye movements. **Methods:** We examined the static and dynamic representation of eye-position in parafoveal V1 of an alert macaque by recording extracellular activity as the animal performed sequences of fixations and saccadic eye movements. To probe population codes for eye position, we recorded from multiple neurons (typically 3-10) simultaneously using a multielectrode array. **Results:** Neurons showed substantial modulations of visually-evoked activity by the position of the eyes in the orbit (i.e. 'gain fields'). We used these tuning functions to decode the eye position from the collected spikes on a trial-by-trial basis, thereby allowing an assessment of the reliability of eye-position representation. We found that the position of the eyes could be predicted to within a few degrees of visual angle during fixation, even using as few as two V1 neurons. Further, the representation of eye position was updated rapidly after the offset of saccades (within 50-100ms). These findings point to a highly reliable and nimble representation of eye position in primary visual cortex. However, because retinal slip had strong effects on evoked activity during the saccade, large errors in eye position representation were observed during the peri-saccadic interval. **Conclusions:** The representation of eye position in V1 is sufficient to support fluid and accurate visuomotor behaviour.

POS-TUE-072

STRUCTURAL AND FUNCTIONAL DEPENDENCE OF SPIKE-COUNT CORRELATIONS IN AREA MT

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Purpose: Shared inputs and reciprocal connectivity cause the spike rates of sensory cortical neurons to fluctuate together ('noise correlations'). The strength of this correlation depends on the functional similarity of the measured neurons and their relative position in the cortical sheet. Here we ask how spike-count correlation depends on relative position and functional similarity of neurons in the middle temporal (MT) area of the visual cortex of a New World primate, the marmoset. **Methods:** Extracellular recordings were made from area MT in sufentanil-anaesthetised marmosets (3 animals; 12 recording sites). An array of 8 electrodes (separation 0.2 mm) was inserted perpendicular to the cortical surface; on each electrode, 8 channels (spacing 0.2 mm) spanned laminae 3-6. Spike-count (multi-unit activity) was measured during 2 s presentation of a large field of dots, drifting in each of 4 directions. We calculated spike-count correlation (Rsc) for all possible pairs of recording sites where we could recover direction sensitive multi-unit activity (n = 1618 pairs). **Results:** Rsc declined monotonically with distance between electrodes and with difference in direction preference, but did not depend on relative laminar position. We also characterised the dependence of Rsc on each pairing of these three dimensions (eg. distance between electrodes and difference in direction preference). In each case the joint distribution was well approximated by a linear combination of the two relevant dimensions (r = 0.66-0.87). **Conclusion:** Noise correlations are strongest for neurons within the same cortical column, particularly when they have similar functional properties.

POS-TUE-073

MAPPING OF THE RETINAL SPECIALISATIONS IN THE NUCLEUS ISTHMI PARVOCELULLARIS OF THE PIGEON *Columba livia*

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Previous studies have shown that the isthmotectal system constitutes a mechanism of visual-spatial attention in the midbrain of vertebrates. In this circuit, the nucleus isthmi parvocellularis (Ipc) sends feedback signals to the optic tectum (TeO) enhancing the transmission of retinal visual inputs from selected tectal locations to higher visual areas. In pigeons, the retino-tectal map has an increased representation of two retinal areas with higher ganglion cell density, the central fovea and the red field. **Purpose**: in this study we proposed to study the reciprocal projections between the Ipc and TeO to elucidate whether the representations of these retinal specialisations are also enhanced in Ipc. **Methods**: we made multiple injections of the retrograde tracer biotinilated dextran amine in TeO (n=21) finding the position of traced neurons in both the Ipc and the retina. Additionally, we performed electrophysiological recordings (n=22) of visually evoked potentials in Ipc and determined the position of their visual receptive fields. Finally, using these data we calculated the magnification factor for each retinal region in Ipc, comparing the distance between two points in Ipc with the distance between their respective loci, in both the retina and the visual field. **Results**: our results show that in the Ipc the retinal specialisations have a significantly higher magnification factor when compared to the periphery of the visual field, indicating that these regions have an enlarged representation in this nucleus. **Conclusion**: these results suggest that the circuit Ipc-Teo may enhance the visual processing of stimuli in all parts of the visual field, especially within regions perceived at high spatial resolution, particularly relevant during behaviours such as foraging and detection of predators.

POS-TUE-074

BIASES IN PERCEPTUAL MEMORY

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Purpose: When an ambiguous visual stimulus is presented intermittently, the percept on one presentation is the same as on the previous presentation more often than not. Our aim was to study biases in this form of perceptual memory. **Methods:**We used binocular rivalry to produce an ambiguous percept. The stimulus consisted of an oblique grating presented to one eye and an orthogonal grating to the other eye, with the sum of the monocular contrasts set equal to one. Gratings were presented for one second, and the eight adult human observers reported seeing one or the other of the monocular stimuli. **Results:** As expected, increasing the contrast of the right eye's stimulus made it more likely that this stimulus would be perceived. Unexpectedly, the right eye contrast required to obtain equal probabilities of the two percepts was substantially less than the left eye contrast. Swapping the grating stimuli between eyes showed that this was a bias towards the right eye, not to a specific stimulus orientation. We also measured the probability of a percept as a function of the previous percept. In accordance with previous work, the percept tended to persist from one presentation to the next. Nulling this bias with a change in contrast allowed us to measure the percept-induced bias in stimulus terms. We have obtained comparable results with another ambiguous stimulus, structure from motion. **Conclusions:** There are two biases in the perceptual memory produced by intermittently presented ambiguous stimuli. One is chronic, and is towards the right eye, and the other is a temporary bias in the direction of the previous percept.

POS-TUE-075

THE EFFECTS OF NEUROTROPHINS AND CHRONIC ELECTRICAL STIMULATION DELIVERED TO THE DEAFENED GUINEA PIG COCHLEA

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Purpose: Spiral ganglion neurons (SGNs) in the deafened cochlea undergo continual degeneration ultimately resulting in cell death. The exogenous application of neurotrophins (NTs) can prevent SGN loss, with the survival effects enhanced by chronic intracochlear electrical stimulation (ES) from a cochlear implant. Furthermore, NT treatment can enhance resprouting of the SGN peripheral processes. However, little is known about the changes to the SGNs that occur following ES and NT treatment. Here we have examined SGNs and their peripheral processes following the delivery of brain derived neurotrophic factor (BDNF) and chronic ES. Methods: Adult guinea pigs (n=14) were deafened with ototoxic aminoglycosides and implanted two weeks later with an electrode array containing a cannula for NT delivery. A clinical device was used to deliver chronic intracochlear ES over a four week treatment period. Cochleae were collected and prepared for examination on a transmission electron microscope. Results: NT treatment was effective in reducing the loss of SGNs and their peripheral processes following deafness. The peripheral processes were significantly larger in NT treated cochleae, with or without ES, compared to cochleae not treated with NTs ($p<0.0005$). Interestingly, resprouting processes were observed within the osseous spiral lamina, the spiral limbus and the scala tympani. Conclusion: This study has shown that NT delivery was effective in reducing the retraction of the SGN peripheral processes that normally occurs following deafness. Process resprouting was also enhanced following NT treatment and processes were observed within the scala tympani. This finding raises the possibility of a direct connection between the SGNs and the electrode array that may improve the nerve-electrode interface.

POS-TUE-076

CONGENITAL DEAFNESS CAUSES CHANGES IN GLYCINE ANATOMY AND PHYSIOLOGY IN BRAINSTEM COCHLEAR NUCLEUS

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Purpose: The cochlear nucleus (CN) is the gateway to central auditory processing. Sound localisation begins in the CN via large excitatory synapses between the auditory nerve and bushy cells. We have demonstrated that deafness can modify the excitatory input. Here we investigate whether congenital deafness can alter inhibition in a well-characterised mouse model for human childhood deafness (*Shaker-2* mice). **Methods:** Hearing thresholds of homozygote (deaf; n=7) and heterozygote (hearing; n=6) *Shaker-2* mice were measured with auditory brainstem responses (ABRs) to multiple sound intensities (10-90 dB SPL) at multiple frequencies (8, 16, 24 kHz) in mice aged 15-70 days. Anatomical changes in glycinergic input were investigated in deaf (n=26) and hearing *Shaker-2* mice (n=28) via immunohistochemistry of the glycine transporter GLYT2 onto spherical and globular bushy cells. Quantification of GLYT2-immunopositive presynaptic terminals was performed in parasagittal sections of the CN using fluorescence microscopy. Physiological changes in glycinergic input in deaf (n=7) and hearing *Shaker-2* mice (n=3) were investigated via miniature glycinergic postsynaptic currents. **Results:** Homozygote *Shaker-2* mice show mean ABRs thresholds > 90 dB SPL at all frequencies and ages tested. Deafness significantly reduced the mean number of GLYT2-positive puncta surrounding globular bushy cells (4 vs. 13 puncta; $P<0.05$) and the mean soma size of globular bushy cells (165 vs. 200 μm^2; $P<0.05$) when compared to normal hearing mice. Deafness decreased mean frequency of miniature glycine events in spherical bushy cells compared to normal hearing mice (0.5 vs. 1.3 Hz; $P<0.05$). **Conclusion:** Our results suggest that the glycine inhibitory circuitry could be dependent on sensory input for development and maintenance.

POS-TUE-077

A 3-DIMENSIONAL MODEL OF FREQUENCY REPRESENTATION IN THE COCHLEAR NUCLEUS OF THE CBA/J MOUSE

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The relationship between structure and function is an invaluable context with which to explore biological mechanisms of normal and dysfunctional hearing. The systematic and topographic representation of frequency originates at the cochlea, and is retained throughout much of the central auditory system. The cochlear nucleus (CN) initiates all ascending auditory pathways so we developed a 3-dimensional tonotopic model to explore possible frequency specializations at this early stage. Prior descriptions of tonotopy in the mouse CN include a 2-D cFos map (Ehret & Fischer, 1991), 3-D maps derived from reconstructions of injection sites (Muniak et al., 2007), and multi-track electrophysiological sampling (Luo et al., 2009). Building upon our previous efforts, we have reconstructed in 3-D the trajectories of labeled auditory nerve (AN) fibers following multiunit recordings and dye injections (n=30) in the anteroventral CN of the CBA/J mouse. We observe that each injection produces a continuous sheet of labeled ascending and descending branches of the CN. Individual cases were combined using 3-D alignment procedures to provide a comprehensive view of the projection pattern of AN fibers in the CN with respect to frequency. AN fibers exhibit a systematic and tonotopic arrangement in each subdivision with a clear separation of isofrequency laminae. The combined dataset was used to derive a 3-D reference model of tonotopy throughout the entire volume of the CN. This model can serve as a tool for visualizing CN tonotopy and as a dataset upon which hypotheses concerning frequency and location in the CN can be tested.

POS-TUE-078

RECIPROCAL CONNECTIVITY BETWEEN THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS AND THE DORSAL COCHLEAR NUCLEUS OF THE CBA/CAJ MOUSE

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Recent observations suggest that descending projections in the brain outnumber ascending ones (Winer and LaRue, 1987; Jones, 2000), but few reports have been published that describe the synaptic organization of these projections. Descending pathways are an essential element of sensory systems, and may facilitate the real-time modification of neural responses to external stimuli at any stage from periphery to cortex. In the auditory system, the cochlear nucleus (CN) is key because it initiates all ascending pathways, including a direct contralateral projection to the inferior colliculus (IC). Studies of descending projections in rat and guinea pig suggest that colliculo-cochlear nucleus projections originating in the central nucleus of the IC (CNIC) terminate bilaterally and topographically in the dorsal CN (DCN, Caicedo and Herbert, 1993; Malmierca et al., 1996). We have confirmed this projection in the CBA/CaJ mouse by using multiunit recordings and dye injections in the CNIC (n=6). The frequency of the IC injection site matches the frequency location in the DCN as determined by a 3-D frequency atlas created for the mouse CN (Muniak et al., 2011). Moreover, by applying anterograde (dextran amines) and retrograde (beta subunit of cholera toxin) tracer injections in CNIC, we were able to demonstrate that anterogradely labeled descending projections form bouton contacts in close proximity to retrogradely labeled cells in the contralateral DCN. This relationship suggests that the descending pathway from CNIC terminates on the same DCN neurons from which the ascending projections originate.

POS-TUE-079

CHRONIC MULTI-UNIT RECORDING FROM CAT AUDITORY CORTEX

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We have previously shown that neonatal profound deafness results in a loss of the normal cochleotopic organization of primary auditory cortex (AI), but that environmentally-relevant chronic electrical stimulation (CES) via a multi-channel cochlear implant can restore that organization. To explore the time course of this re-establishment of cochleotopy, we are developing procedures to obtain chronic recordings of multi- and single-unit responses from the AI of cats over extended periods. We have evaluated the stability of AI recordings over time by recording responses to tonal stimuli in two normal-hearing cats. In each cat, a planar silicon electrode array (Blackrock Microsystems; Salt Lake City, Utah) was implanted in putative AI under sterile conditions. Initial recordings from the arrays were made approximately 4 weeks post-surgery and subsequent recordings at approximately 2-week intervals, with the cats either gently restrained or anesthetized with Xylazil (2mg/kg, s.c.) and Ketamine (20mg/kg, i.m.). Calibrated tone-burst stimuli were presented via a free-field speaker positioned approximately 10 cm from the ear contralateral to the implanted cortex. Responses to tonal stimuli were observed on 49 of 90 channels (54%) in the initial recording sessions and on 37 of the 90 channels (41%) in the final recording session (4 and 5 months later in the two cats). At 10 recording sites at which a reliable frequency-intensity response area could be recorded over this period there was no significant difference in either characteristic frequency (paired t-test; $p = 0.68$) or threshold ($p = 0.70$). This stability over time indicates that these recording procedures are appropriate to future studies of the time course of the effects of deafness and CES on cochleotopic organization.

POS-TUE-080

A PREVENTION AND TREATMENT TRIAL OF ALPHA LIPOIC ACID ON NOISE INDUCED HEARING LOSS

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Purpose: There are no effective treatments for Noise-Induced Hearing Loss (NIHL), however, recent studies have shown that certain antioxidants can, to some extent, protect against NIHL. There is some evidence to suggest a link between NIHL and oxidative stress, and antioxidants may play a role in reducing the damaging effects of reactive oxygen species within the peripheral auditory system. Methods: Our study measured changes in hearing thresholds following mild acoustic trauma in animals given Alpha Lipoic Acid (ALA) (100mg/kg), a potent antioxidant, either before or after the acoustic trauma. Male Long Evans rats (n = 16) were separated into 4 groups; pre-ALA (n = 5); 1 week of ALA injections followed by NIHL, pre-saline (n = 5); 1 week of saline injections followed by NIHL, post-ALA (n = 3); NIHL followed by 2 weeks of ALA injections and post-saline (n= 3); NIHL followed by 2 weeks of saline injections. The mild acoustic trauma consisted of a 16 kHz bandpass (1/10th octave noise (112 dB SPL)) for 50 seconds. The hearing thresholds of the rats in each group were assessed by recording Auditory Brainstem Responses at 0, 4, 8, 16, 32 and 64 days following NIHL, The procedures were approved by the Animal Ethics Committee of UWS (A9201). Results: We found that hearing thresholds in rats pre-treated with ALA recovered faster than control (pre-saline treated) rats, while the post treatment with ALA had no effect on threshold recovery rates compared to control (post-saline treated) rats. Conclusion: Our results suggest that antioxidants taken prior to acoustic trauma may assist in the rate of recovery of hearing thresholds after NIHL.

POS-TUE-081

TAURINE: A FREE RADICAL SCAVENGER THAT MITIGATES NOISE-INDUCED HEARING LOSS

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Purpose: Exposure to acoustic trauma causes an increase in metabolic activity in the inner ear, resulting in free radical production. Free radicals such as nitric oxide (NO) can cause cellular damage by attacking proteins, lipids, and DNA, which in turn can cause apoptosis and necrosis. For this study, we tested if taurine, a potent NO scavenger, could mitigate noise-induced hearing loss (NIHL). The dose response relationship of taurine was investigated physiologically and anatomically. **Methods:** Male CBA mice aged 4-10 weeks (n=60) were randomised into six groups (n=10 per group) treated with 0.9% saline (control) or taurine (50, 100, 200, 300 or 400 mg/kg) via daily IP injections over 14 days (7 days before and after acoustic trauma). Acoustic trauma was 8-24 kHz banded noise at 110 dB SPL for 2 hrs. Auditory brainstem responses were tested at 8, 16 and 24 kHz, and collected one week before, one week after, and one month after acoustic trauma to record pretreatment hearing threshold levels, temporary threshold shifts (TTS), and permanent threshold shifts (PTS), respectively. The anatomical effect of taurine on hair cell survival was investigated with cytocochleograms using succinate dehydrogenase histochemistry. **Results:** Taurine significantly attenuated the effects of acoustic trauma as shown by TTS and PTS when compared to saline controls ($P<0.05$ at 8, 16 kHz for TTS and 8, 16, 24 kHz for PTS). Thresholds shifts were on average 13.2 dB better in all taurine treated mice compared to the saline control group. A taurine dose of 200 mg/kg yielded the greatest effect in mitigating against NIHL compared to saline controls ($P<0.05$ at 8, 16, 24 kHz for TTS and PTS). Preliminary observations suggest taurine improved hair cell survival when compared to saline-treated mice. **Conclusion:** Taurine is a safe anti-oxidant, and a common component in normal diets and commercial energy drinks. Our future studies will investigate taurine's mechanism of action and its potential as a therapeutic agent to protect against NIHL.

POS-TUE-082

GENE THERAPY AFTER HEARING LOSS FOR LONG-TERM NEURAL PROTECTION

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Introduction: The administration of exogenous neurotrophins (NTs) to the deafened cochlea via mini-osmotic pumps can promote spiral ganglion neuron (SGN) survival and peripheral fibre regrowth. However, the duration of exogenous NT delivery using such a technique is finite and this protective effect has not been shown beyond two weeks after cessation of NT administration, indicating that long-term NT treatment is required for sustained benefits. **Purpose:** Using gene therapy techniques, we aim to provide a sustained source of NT to the deafened cochlea localised to the organ of Corti for long-term SGN survival. **Methods:** Adenoviral-based NT-gene therapy vectors were injected into the lower basal turn scala media of ototoxically-deafened guinea pigs. The resulting gene expression profiles, effects on SGN survival and peripheral neurite outgrowth were analyzed after seven and eleven weeks of treatment. **Results:** Adenoviral vectors injected into the scala media remained localized within this small region, limiting an immunogenic response and allowing for more extended expression than is possible with adenoviral gene therapy in other tissues. After a single NT-gene therapy inoculation there was a significant increase in SGN survival in the basal turn of the cochlea (proximal to the injection site) at both seven weeks ($n = 5$, 1.3-fold increase) and eleven weeks ($n=5$, 1.5-fold increase) post inoculation. These increases were statistically different in comparison to contralateral, non-injected cochleae ($p<0.05$). **Conclusion:** Gene therapy can enable sustained SGN protection after deafness, following a single injection of adenoviral vectors into the scala media.

POS-TUE-083

A PUZZLING LACK OF CLAUSTRUM PROJECTIONS TO MARMOSET AUDITORY CORTEX

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Background: The claustrum is a thin sheet of neurons inserted between the insular cortex and the basal ganglia. Very few studies have addressed the anatomy and physiology of this part of the brain. Nonetheless, the claustrum has become the subject of intense speculation regarding a potential role as regulator and synchronizing element for higher cortical functions. This hypothesis is largely based upon the observation of dense projections from the claustrum to most cortical areas so far studied. Here we examined the pattern of connections between the claustrum and the auditory neocortex of the marmoset monkey. **Methods:** Neuroanatomical tracer injections were placed in several auditory areas of 7 adult marmosets, under alfaxan (10 mg/kg) anaesthesia. For comparison, tracer injections into various other frontal, parietal and temporal cortical areas were placed in the same animals. **Results:** Surprisingly, the marmoset claustrum exhibited very limited or no connections with primary (core), secondary (belt) or even higher order (parabelt) auditory areas. Clear indications of tracer movement to distant sites (thalamus, prefrontal cortex) were observed from the same injections, and injections into non-auditory cortical areas of the same animals yielded copious label in the claustrum. **Conclusion:** These results suggest a more complex pathway for auditory information to the claustrum in primates compared to other studied species, and challenge the notion that the claustrum is a connection hub capable of coordinating low-level sensory and cognitive information across the entire cerebral cortex.

POS-TUE-084

SYNERGISTIC EFFECT OF MAGNESIUM SULPHATE AND N-ACETYL-L-TRYPTOPHAN IN AMELIORATING BLOOD-BRAIN BARRIER OPENING IN CLOSED HEAD TRAUMA

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Purpose: Neurogenic inflammation and substance P (SP) release and binding to NK1 receptors have been shown to play a pivotal role in opening of the blood-brain barrier (BBB) and formation of cerebral oedema in closed head trauma. Magnesium sulphate ($MgSO_4$) and the NK1 receptor antagonist, N-Acetyl-L-tryptophan (NAT), have been shown to reduce brain oedema and improve motor outcome when administered separately. This study investigated, in the short term, possible synergistic effects of their combined administration in a rodent model of closed head trauma. **Methods:** Under general isoflurane anaesthesia, male Sprague-Dawley rats (380-420g) were injured using a weight drop model, and the permeability of the BBB to Evans blue (EB) was assessed at 5h and 24h post-injury in treated groups using single agents, ($MgSO_4$, 30.1mg/kg, I.V.) or NAT (2.463mg/kg, I.P.) or both agents (n=6/group/time point) compared to a non-treated group (n=6/time point) and a non-injured sham control group (n=6). All treatments were administered within 5 minutes after trauma. Thirty minutes prior to saline perfusion EB was injected I.V. (4% 1mL/kg). Perfused brains were divided sagittally and each hemisphere weighed then scanned using a Xenogen IVIS 100 imaging system to measure EB autofluorescence at light excitation wavelength of 615-665nm. Each hemisphere was then homogenised in trichloroacetic acid to extract EB. The supernatant was measured for EB absorbency concentration using a spectrophotometer at 620nm excitation. **Results:** When jointly administered $MgSO_4$ and NAT produced a significant ($p<0.05$) reduction in BBB permeability to EB at 24h after trauma, but a synergistic effect was not noted at 5h post-trauma. **Conclusion:** Long-term benefit of combined treatment is suggested and merits further investigation with repeated daily treatment.

POS-TUE-085

IDENTIFICATION OF NEDD-4 MEDIATED UBIQUITINATION IN HUMAN BRAINS AFTER TRAUMATIC BRAIN INJURY (TBI)

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Purpose: Traumatic Brain Injury (TBI) is the leading cause of death and disability in children and young adults and places an extraordinary financial burden on the entire community. Beyond surgical intervention there is no cure for TBI, however since many neurons take days to die after trauma there is a window of opportunity to improve patient outcomes. Previously we have found in rodent models that Ndfip1, an adaptor for Nedd-4 (an E3 ubiquitin ligase) is a neuroprotective protein upregulated after TBI. This study aims to further define the role of Ndfip1-mediated neuroprotection in human TBI. **Methods:** Ndfip1 expression has been studied using immunohistochemical analysis of post-mortem TBI brains obtained from subjects who have died at different time-points. Cerebrospinal fluid (CSF) samples from TBI patients have also be investigated through western blot analysis to examine whether Ndfip1 levels are higher in TBI patients compared to non-TBI patients. **Results:** Ndfip1 has been detected in post-mortem brains with higher Ndfip1 levels detected in trauma brains compared to non-trauma brains. Ndfip1 has also been detected in human CSF (n=5) and TBI patients demonstrate higher levels of Ndfip1 compared to non-TBI patients. **Conclusion:** Preliminary data suggests that Ndfip1 is present in human post-mortem cortex and post-TBI CSF, however further work is needed to determine whether Ndfip1-mediated neuroprotection is active in humans after TBI. Future work will also explore the possibility that Ndfip1 in the CSF can be utilised as a TBI biomarker for patient outcome and recovery.

POS-TUE-086

PROCESSING CHANGES IN SENSORY CORTEX FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

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Background: Traumatic brain injury (TBI), as occurs in car accidents or from falls, can result in persistent sensorimotor and cognitive deficits. Long-term human studies suggest that a likely major contributor to these deficits is altered sensory processing but there are few animal models of sensory processing in TBI. We investigated diffuse TBI-induced changes in neuronal responses in the cortex that processes input from the main sensory system in rats, the large facial whiskers. **Methods**: TBI (n=15) was induced using the weight-drop impact acceleration method which involves dropping a 450g weight from 2 meters onto an anaesthetized animal's skull; sham controls received surgery only (n=10). Animals were tested for sensorimotor function prior to and after injury. At 8-10 weeks post-trauma, in terminal experiments, extracellular recordings were obtained from barrel cortex neurons in response to whisker motion, including motion that mimicked whisker motion in awake animals undertaking different tasks. **Results**: There were significant sensorimotor deficits up to 6 weeks post-TBI. In cortex there were lamina-specific neuronal response alterations that appeared to reflect local circuit changes. In [input] Layer IV or [output] Layer V there were no significant differences between TBI and Sham cells in response patterns or rates to any stimuli. In Layers II and Upper and Deep III, involved in intra-areal processing and long-range integration, TBI significantly increased responses to all stimuli, ranging from those modelling exploratory behaviour through to discrimination behaviours. **Conclusion**: TBI induces long-term directional changes in integrative sensory cortical layers and these changes may contribute to sensorimotor deficits post-trauma.

POS-TUE-087

MOTOR CORTEX NEUROPLASTICITY FOLLOWING BRACHIAL PLEXUS TRANSLOCATION

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Purpose: Brain plasticity describes the ability of the CNS to modify its structural organisation as an adaptive response to functional demand and research has demonstrated that brain plasticity does indeed continue on into adulthood. As a consequence, brain function in adults remains adaptable and can change in response to peripheral events such as amputation. We report the case of a 27 year old woman who suffered complete avulsion of her left brachial plexus and amputation of her right arm following an accident. The right brachial plexus, remained functional and was translocated to her intact left arm so that it might provide a basis for future function. Plastic changes in sensorimotor cortex were studied to derive correlations between instinctiveness of movement and level of fMRI changes as possible forerunners for permanent functional cortical rearrangement. Methods: Using fMRI, changes in the sensorimotor cortex were investigated through left elbow-flexion and finger-tapping. Results: Cortical activation of the left hemisphere was maintained between both tasks reflecting incorporation of the right brachial plexus into the circuitry now innervating the left upper limb. Significant cortical activation of the contralateral hemisphere was also present and more pronounced during elbow-flexion compared to finger-tap and correlated with the patient's subjective feeling that elbow-flexion had become second-nature. Conclusions: Neuronal re-organisation does occur over the medium term in adults following brachial plexus translocation. The degree of cortical rearrangement correlated with subjective ease of movement. This allows us to hypothesise as to the effects within the cortex as the brain adapts to the new function of the right brachial plexus and possible changes in its self-attribution of the left arm.

POS-TUE-088

A HYPOXIC INSULT IN PATIENTS WITH TRAUMATIC BRAIN INJURY ENHANCES CEREBRAL INFLAMMATORY CYTOKINES, SERUM BIOMARKERS AND BLOOD-BRAIN BARRIER DYSFUNCTION ASSOCIATED WITH UNFAVOURABLE OUTCOME

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Objectives: A hypoxic insult following traumatic brain injury (TBI) has been long recognised as a major contributing factor to adverse outcomes in humans. To elucidate the mechanisms leading to aggravated brain damage, we embarked a study on patients with severe TBI to examine differences in hypoxic/normoxic individuals in cerebral inflammation, blood levels of brain injury markers, blood-brain barrier (BBB) dysfunction and how these pathological changes relate to neurological outcome at 6 months. Methods: 42 TBI patients were recruited following admission to the Alfred Hospital in Melbourne, Australia. Inclusion criteria were Glasgow Coma Scale ≤9, and an extraventricular drain (EVD) for monitoring the intracranial pressure (ICP) and release of cerebrospinal fluid (CSF). Neurological outcome was assessed at 6 months using the Glasgow Outcome Scale Extended (GOSE). Paired CSF and blood serum were collected daily from the day of admission (day 0) to day 5 post-injury. 22 hypoxic (Hx) TBI patients had SaO2<92% at the scene whilst the remaining normoxic (Nx) 20 patients had a normal SaO2. Eight cytokines were measured in CSF for 6 consecutive days by multiplex ELISA, while albumin in CSF and serum, and S100, NSE and MBP in serum by conventional ELISA. The blood-brain barrier dysfunction was assessed daily by calculating the CSF/serum albumin quotient. Results: Overall, the cytokines GM-CSF, IFN and to a less extent TNF, only increased in CSF of Hx, but not in Nx patients. These differences were particularly observed at days 4-5 post-TBI-Hx. Other cytokines increased similarly in both groups, excluding IL-8 which was not significantly elevated in either cohort. When Hx and Nx patients were compared, MBP and S100 were more elevated in the Hx cohort but all three markers MBP, S100 and NSE were significantly higher in those Hx patients with unfavourable outcomes at 6 months post-injury (GOSE 1-4). Interestingly, over the 6 days analysed, the prolonged elevation of injury markers in serum of Hx patients coincided with a sustained dysfunction of the BBB on days 3 to 5. Conclusions: Altogether this study indicates that hypoxia following TBI enhances secondary brain damage likely by exacerbating neuroinflammation and BBB permeability. These changes were associated with a more robust biomarker extravasation in peripheral blood, and adverse neurological outcomes.

POS-TUE-089

DEFICIENCY OF THE CHEMOKINE MCP-1 IMPEDES THE RECRUITMENT OF SUBVENTRICULAR ZONE NEUROBLASTS INTO THE PERICONTUSIONAL CORTEX AFTER TRAUMATIC BRAIN INJURY IN ADULT MICE

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Purpose: Neurogenesis is stimulated following injury to the adult brain and could potentially contribute to tissue repair. Evidence from studies on stroke models suggests that the chemokine monocyte chemoattractant protein-1 (MCP-1), which is released by activated glia and infiltrating macrophages at the site of injury, plays a role in the recruitment of subventricular zone (SVZ) neuroblasts towards the damaged tissue. The aim of this study was to determine firstly whether MCP-1 plays a similar role in neuroblast migration after traumatic brain injury (TBI), and secondly, whether MCP-1 affects other stages of injury-induced neurogenesis. **Methods:** MCP-1 deficient(-/-) and wild-type(wt) mice were subjected to a closed head injury model of TBI or sham-operation and were killed at 1,2,4&6 weeks (n=2-10). Mice were administered bromodeoxyuridine (BrdU; 200 mg/kg i.p.) on days 1-4 to label proliferating cells. Brains were immunolabelled to detect BrdU, doublecortin(Dcx) and NeuN-positive cells, to assess changes in neurogenic stages including proliferation, neuronal differentiation, neuroblast migration and long-term survival. Labelled cells were quantified in the SVZ and the pericontusional cortex, as well as in the hippocampal dentate gyrus(DG). **Results:** TBI-induced proliferation in the DG and SVZ was not affected in MCP-1-/- mice, nor was the proportion of new cells that become mature neurons in the DG or cortex ($P>0.05$). However, 40% fewer Dcx+ neuroblasts migrated from the SVZ to the pericontusional cortex in the MCP-1-/- mice compared to wt ($P<0.05$). **Conclusion:** Our data suggest that MCP-1 recruits neuroblasts to the injured cortex after TBI; however, this does not result in an ultimate increase in new neurons.

POS-TUE-090

THE NEURONAL SEROTONIN TRANSPORTER DOES NOT TRANSLOCATE FROM THE CELL MEMBRANE TO THE CYTOSOL FOLLOWING NEONATAL BRAIN INJURY

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Purpose: Following neonatal hypoxia-ischemia (HI) serotonin (5-hydroxytryptamine, 5-HT) levels are decreased in the brain. The serotonin transporter (SERT) is critical for the regulation of the brain 5-HT and the efficient reuptake of 5-HT is dependent on the localisation of SERT in the pre-synaptic cell membrane. However SERT can also traffic away from the cell membrane into the cytosol and, after injury, may partly contribute to HI-induced decreases in 5-HT levels. In addition, there is contradictory evidence to suggest that glial cells may contribute to the clearance of 5-HT after brain injury. Methods: Using a postnatal day 3 (P3) HI rat pup model (right carotid ligation + 30 min 6 % O_2), we examined the localisation of SERT in the cell membrane and cytosol of control (n=6) and P3 HI (n=6) rat pups on P10. To determine the potential localisation of SERT on glial cells we performed dual immunofluorescent labelling. Results: In both control and P3 HI animals we found that SERT was retained on the cell membrane and is not harboured in the cytosol. In addition, SERT was only localised on neurons. We did not find evidence for the co-localisation of SERT on microglia nor astrocytes. Conclusions: Neuronal membrane SERT is the primary regulator of synaptic 5-HT availability in the intact and P3 HI-injured neonatal brain. Since concomitant reductions in 5-HT, SERT and serotonergic neurons occur after neonatal HI, it is plausible that the decrease in brain 5-HT is a consequence of SERT being lost as neurons degenerate.

POS-TUE-091

AFFERENT AND EFFERENT NEURAL CONNECTIONS OF THE DORSAL RAPHE NUCLEI ARE DISRUPTED AFTER NEONATAL BRAIN INJURY

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Purpose: Neonates exposed to a hypoxic-ischemic (HI) insult may suffer life-long neurological deficits although the central mechanisms underpinning these deficits are not clear. Serotonergic neurons in the dorsal raphe nuclei in the brainstem are lost after neonatal HI. However, the brainstem is located outside the primary regions of injury in the forebrain and does not endure local changes in blood flow after HI. We hypothesised that brainstem raphe neurons may be lost after neonatal HI because neural connections between the forebrain and raphe nuclei may be disrupted and compromise the survival of serotonergic neurons. **Methods:** On post-natal day 3 (P3), HI was induced in rat pups (right common carotid artery ligation+30 min 6% O_2). Five weeks after injury a retrograde tracer, choleratoxin b (CTb), was injected into the motor cortex (control, n=7; P3 HI, n=7) or specific dorsal raphe nuclei (control, n=4; P3 HI, n=4). Brains were then analysed to identify CTb-positive, retrogradely-labelled neurons. **Results:** Following injections into the motor cortex we found a significant reduction in numbers of retrogradely-labelled neurons in specific raphe nuclei in HI-injured animals compared to controls. Numbers of descending projections from forebrain nuclei to the dorsal raphe were also reduced in HI animals compared to controls. **Conclusion:** Disruption of both afferent and efferent neural connections between forebrain and raphe nuclei may contribute to losses of remote raphe serotonergic neurons observed after P3 HI. The interruption of these pathways may also constitute a mechanism of neuronal loss underpinning neonatal HI-induced deficits.

POS-TUE-092

BRAIN INFLAMMATION AND INJURY RESULTING FROM CHORIOAMNIONITIS IS EXACERBATED BY HIGH TIDAL VOLUME RESUSCITATION IN PRETERM LAMBS

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Purpose: We investigated whether inflammation *in utero* exacerbated abnormal cerebral haemodynamics, brain inflammation and injury after high tidal volume (V_T) resuscitation. **Method:** Intra-amniotic lipopolysaccharide (LPS) was administered to preterm lambs (~126 days gestation; term 147 days) 2 days prior to delivery and resuscitated using an: 1) injurious, a V_T of 12 mL/kg for 15 min followed by a V_T of 7 mL/kg for 75 min (LPS_{INJ}, n=5); or 2) gentle, surfactant (480 mg Curosurf, Chiesi) a 20 second sustained inflation followed by a V_T of 7 mL/kg for 90 min (LPS_{GENT}, n=7) resuscitation strategy. Physiological measurements were obtained using indwelling flow-probes and catheters, echocardiography and near infrared spectroscopy. Brains were collected for histological, immunohistochemical and molecular assessment compared to unventilated controls (LPS_{UVC}, n=6 and SAL_{UVC}, n=6). **Results:** Oxygenation, lung compliance and cerebral tissue oxygenation index were lower in LPS_{INJ} lambs compared to LPS_{GENT}. Relative to SAL_{UVC}, LPS_{UVC} lambs had increased brain inflammation (IL-1β, IL-6 and IL-8 mRNA expression; $p<0.05$), increased blood brain barrier permeability and decreased cerebral vessel density ($p=0.08$) and markers of angiogenesis (Flk-1 mRNA expression; $p=0.005$). Resuscitation further amplified inflammatory mRNA expression (IL-6 & IL-8 mRNA; $p<0.05$), recruitment of inflammatory cells into the subcortical white matter and blood brain barrier permeability, with the most pronounced increase in LPS_{INJ} lambs. **Conclusions:** Acute intrauterine inflammation causes brain inflammation and injury, which is exacerbated by injurious resuscitation.

POS-TUE-093

FLUID SHIFTS IN THE RAT BRAIN AFTER ISCHAEMIC STROKE

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Purpose Raised intracranial pressure (ICP) is a complication of ischaemic stroke that increases neurological deficit and mortality. It is known that any increase in intracranial volume leads to an increase in ICP. Identification of changes in the main fluid compartments of the brain (cerebral blood volume, tissue fluid volume and cerebrospinal fluid) will further our knowledge of the causes of raised ICP after ischaemic stroke. We aimed to investigate cerebrospinal fluid (CSF) production, and oedema volume in vivo in rat experimental stroke. **Methods:** Middle cerebral artery occlusion (MCAo) was induced in 6 male Wistar rats (3 x 3hr MCAo, 3 x control). A novel contrast-enhanced CSF flow CT scanning method was used to quantify CSF production (total cerebral aqueduct flow) and oedema volume (between-hemisphere difference). CBV was calculated using acute and follow-up perfusion CT. **Results:** CSF aqueduct flow 24 h after ischaemic stroke was 1.04±0.142 µl/min, versus 0.47±0.116 µl/min in controls (P=0.006). Oedema volume in the ipsilateral hemisphere was 50 ± 20.0 µl versus 0±11 µl in controls (P=0.01). There was a strong correlation between CSF flow and ICP (r^2=0.82, P=0.01). **Conclusions:** For the first time in an acute brain injury model, we have shown a dramatic increase in CSF production 24 hours post-injury. If not accompanied by a similar dramatic increase in CSF absorption, this would increase CSF volume and provides a possible explanation for the dramatic increase of ICP. These exciting results suggest a novel avenue of investigation for new therapeutics to treat ICP elevation, which is responsible for a significant burden of disease in stroke and other forms of acute neurological injury.

POS-TUE-094

THE EFFECTS OF THERAPEUTIC HYPOTHERMIA ON INTRACRANIAL PRESSURE AFTER EXPERIMENTAL ISCHEMIC STROKE

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Purpose: Elevation of intracranial pressure (ICP) resulting in further injury is a significant problem in stroke and other forms of brain injury yet current therapies are often inadequate to control elevated ICP. Therapeutic hypothermia is the only neuroprotective therapy with proven benefit in human brain ischaemia. No experimental study to date has determined the effect of hypothermia on ischaemic stroke. Clinical studies in a range of conditions have all used 12-24 hours or longer of hypothermia, and often encounter systemic problems. Experiments investigating the effect of short duration hypothermia are described. **Methods:** Middle cerebral artery occlusion (MCAo) surgery was performed on 22 male Wistar rats. Animals were randomised to normothermic or hypothermic groups (+ 2 sham controls) and ICP was measured using a solid state catheter. Hypothermic animals were cooled to 32.5 °C for 2.5 hours and rewarmed to 37 °C over a further 2.5 hours. ICP and infarct volumes were assessed at 24 hours. **Results:** Pre-stroke ICP was 6 ± 2.3 mmHg. At 24 hours, mean ICP was 31.6 ± 9.3 mmHg ($p < 0.0001$) in normothermic animals, but remained at 7 ± 2.8 mmHg in hypothermic animals. Infarct volume was significantly smaller in the hypothermic group, 15 ± 11 mm^3 compared to the normothermic group, 31 ± 18 mm^3 ($p < 0.05$). **Conclusion:** Short duration (2.5 hours) moderate hypothermia induced soon after stroke completely prevented delayed elevation of ICP in rats. The magnitude of this effect has potential implications for the use of therapeutic hypothermia to treat elevated ICP after stroke and to revolutionise, simplify and extend its application to treat a wider range of neurological diseases.

POS-TUE-095

LPA RECEPTOR DISTRIBUTION AND EFFECT OF INHIBITION IN THE RAT BRAIN FOLLOWING ENDOTHELIN-1 INDUCED STROKE

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Lysophosphatidic acid (LPA) is a bioactive signaling lipid and a modulator of inflammation. Following damage to both mouse and human central nervous system, LPA levels increase where the blood brain barrier is damaged and during inflammation. Localisation of LPA receptors in the rat brain, or their effects during stroke, has yet to be examined. **Purpose**: To investigate the distribution and cellular localisation of LPA receptors in the normal and stroke affected rat brain and investigate the effect of blocking specific LPA receptors after stroke. **Methods**: The middle cerebral artery was constricted by endothelin-1 in conscious rats. Neurological and histological outcome was assessed by neurological deficit score and MCID image analysis. For LPA receptor distribution studies, rats were recovered for 3, 7 or 14 days (n=2/group). For antagonist treatment studies, the $LPA_{1,3}$ inhibitor Ki16425 (1 mg/kg, i.p. n=7) or vehicle (10% DMSO in Na_2CO_3, n=7) was given 1 h post-stroke and daily for 7 days. **Results**: LPA 1-3 receptor immunofluorescences were examined in slide mounted forebrain sections co-incubated with antibodies for glial fibrillary acidic protein, neurons, macrophage/activated microglia, mylinated fibers and blood vessels. LPA_1 appeared to co-localise with fibers and blood vessels in both normal and stroke affected brain, whilst LPA_2 and LPA_3 were co-localised with neurons. Post-stroke treatment with Ki16425 significantly reduced neurological deficits in comparison to vehicle treatment (P<0.05 two way ANOVA) and appeared to reduce damage in both the cortex and striatum, although not statistically significant. Ki16425 treatment had no effect on astrogliosis or microglia activation 7 days after stroke. **Conclusion**: LPA receptors are differentially expressed in rat brain, are not associated with inflammatory cells, but may still be a target for preventing stroke injury through alternate mechanisms.

POS-TUE-096

NEUROPROTECTIVE ACTIONS OF HYPOXIA MIMETIC COMPOUNDS IN A NEONATAL RAT MODEL OF HYPOXIC ISCHEMIC BRAIN INJURY

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Preconditioning or pre-injury treatment with compounds which mimic the effects of hypoxia has been shown to protect the brain against hypoxic-ischemic (HI) insults. Here we have investigated the neuroprotective potential of post-injury treatment using a number of hypoxia mimetics including desferoxamine (DFX, 200mg/kg, n=14), cobalt chloride ($CoCl_2$, 60mg/kg, n=14) and ethyl-3,4-dihydroxybenzoate (EDHB, 200mg/kg, n=16) in a neonatal rat model of HI brain injury. 7-day-old Sprague-Dawley rat pups underwent unilateral common carotid artery ligation in combination with 3 hrs at 8% oxygen. This combined HI procedure results in a significant reduction in ipsilateral hemispheric volume. Immediately following the HI procedure, animals were injected subcutaneously with hypoxia mimetics. Brains were removed 1 week post-injury for histological analysis. DFX, $CoCl_2$ and EDHB treatments were all shown to reduce the degree of brain damage when compared with normoxic littermate controls following HI injury; with a corresponding 39%, 40% and 63% reduction in lesion size assessed by histological staining ($p<0.05$). To examine neuronal loss, NeuN immunohistochemistry and regional brain area analysis was performed. Treatment with DFX resulted in 52%, 31% and 54% reductions in cortical, hippocampal and striatal neuronal loss, respectively. $CoCl_2$ treatment failed to significantly reduce neuronal loss in cortical, hippocampal and striatal tissue compared to normoxic controls. EDHB prevented cortical, hippocampal and striatal neuronal loss of 102%, 115%, and 63% respectively when compared to normoxic controls. These results demonstrate the neuroprotective potential of treatment with hypoxia mimetics following HI brain injury and support their potential use in the clinical treatment of brain injuries.

POS-TUE-097

THE EFFECT OF HYPOXIA-ISCHAEMIA AND SEIZURES ON THE $GABA_A$ RECEPTOR α_1, α_2 AND α_3 SUBUNIT PROTEIN EXPRESSION IN THE NEONATAL PIGLET

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Purpose: Treatment of seizures after neonatal hypoxia-ischaemia (HI) is one of the few therapeutic options available. The principal function of the GABA system in mature brain is inhibition, however in the neonatal brain GABA provides much of the excitatory drive. Whilst anticonvulsants augment GABA's inhibitory actions in mature brain, administration of GABAergic drugs to neonates may exacerbate seizures and worsen HI brain injury. Differences in $GABA_A$ receptor expression will influence receptor pharmacology. We aimed to assess changes in protein expression of the $GABA_A$ receptor subunits in the HI piglet model. Methods: Piglets (n=58) were subjected to a 30min HI insult and euthanased at 24 and 72h. HI animals were grouped based on presence or absence of seizure activity. Cortical brain tissue was collected and $GABA_A$ receptor α_1, α_2 and α_3 protein expression levels analysed by western blot. Results: $GABA_A$ α_1, α_2 and α_3 protein expression was altered following HI. At 24h there were no significant differences subunit expressions between HI, HI-seizure and control animals. At 72h α_1 and α_3 expression was reduced in the all cortices of HI animals when compared with controls. Increased α_2 expression was observed in the temporal cortex of HI animals, and reduced in frontal, parietal and occipital cortices when compared with controls. Conclusions: By 72h the $GABA_A$ receptor α_1, α_2 and α_3 subunit expression was significantly altered following neonatal HI; presence of seizures further altered expression levels. Knowledge of which $GABA_A$ receptor subunits are abundant is critical to develop effective seizure treatment strategies specific to the neonatal brain.

POS-TUE-098

COMPARISON OF METHODS FOR THE ISOLATION OF MICRORNA FROM RAT AND HUMAN PLASMA: BEGINNING THE SEARCH FOR BIOMARKERS OF NEUROLOGICAL DISORDERS

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Purpose: MicroRNA are small non-coding RNA that regulate gene expression. Recently, microRNA have been identified in the circulatory system where their expression profiles can be indicative of disease states including those with a neurological basis. However, because of the low levels of microRNA in circulation it is possible that erroneous data may be derived if RNA isolation and analysis are not optimal. **Methods**: Here, we compare the isolation of microRNA from 200 µl rat (n=3) and human (n=3) plasma samples using three commonly used extraction protocols; MirVana PARIS, Norgen total RNA columns, or TRIzol paired with the Norgen column. Exiqon RT-qPCR was used to determine extraction sensitivity by analysing the expression of 2 microRNA known to be found within plasma (Mir-16, Mir-122), and Mir-195, enriched in the brain. **Results**: A three-way, mixed (between-within) ANOVA revealed a significant effect of extraction method (F(2,8)=24.43,p=0.00). The TRIzol extraction method proved to be the least sensitive when compared to MirVana (p=0.05) and Norgen (p=0.001) while the MirVana and Norgen extraction protocols produced comparable levels of sensitivity (p=0.10). Furthermore, a significant effect of microRNA was observed (F(1.0,8)=50.50,p=0.002), with Mir-16 showing significantly higher expression levels than both Mir-122 (p=0.03) and Mir-195 (p=0.004); while Mir-122 showed a significantly greater expression level than the brain enriched Mir-195 (p=0.00). **Conclusion**: Here we show that both Norgen and MirVana extraction protocols provide increase sensitivity over a TRIzol method and would be more suitable for screening plasma for circulating biomarkers indicative of neurological abnormalities.

POS-TUE-099

BMP SIGNALING IS INCREASED IN OLIGODENDROCYTES IN CHRONIC DEMYELINATED LESIONS

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Purpose: One hallmark of demyelinating diseases of the central nervous system is oligodendrocyte apoptosis. This causes a loss of myelin around axons leading to neuronal impairment. Oligodendrocyte regeneration by endogenous oligodendrocyte progenitor cells (OPCs) occurs naturally but is limited in chronic demyelinated lesions. Bone morphogenic protein (BMP) signaling inhibits oligodendrogliogenesis *in vitro* and is increased in acute demyelinated lesions. We have recently found that infusion of Noggin, an endogenous antagonist of BMP4, promotes differentiation during recovery from acute cuprizone-mediated demyelination. Here, we report the changes in the OPC population and in BMP signaling during chronic cuprizone-induced demyelination. **Methods:** We used up to 12-weeks cuprizone challenge to induce chronic demyelination and assessed timepoints during demyelination as well as 2-week and 4-week recovery. Osmotic mini-pumps were used to infuse vehicle or Noggin during chronic cuprizone challenge. Lineage specific proteins and BMP signaling were detected in tissue sections using immunohistochemistry. **Results:** We found that oligodendrocytes were maintained in chronic (10-week) demyelinated lesions (1047±100.5 cell/mm^2) and that 45.2±10.4% of oligodendrocytes within the lesion were positive for pSMAD 1/5/8 (n=3,3). We also found that PDGFRα positive OPCs were maintained within chronic demyelinated lesions (Unchallenged=81.1±20.6 cell/mm^2; Cuprizone = 8.3±17.0 cell/mm^2; p=0.99; n=3,3). **Conclusions:** These initial findings suggest there are significant numbers of OPCs within chronic demyelinated lesions, and that there are significant numbers of oligodendrocytes with active BMP signaling in these demyelinated lesions. We are currently assessing whether inhibiting BMP signaling in these chronic cuprizone demyelinated lesions, which do not remyelinate and are more reflective of chronic lesions of established Multiple Sclerosis, promotes oligodendrocyte maturation and remyelination.

POS-TUE-100

A MULTIPLE SCLEROSIS-ASSOCIATED SINGLE NUCLEOTIDE POLYMORPHISM IN THE MERTK RECEPTOR IS CORRELATED WITH ALTERED MERTK EXPRESSION

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Background: Multiple sclerosis (MS) is a complex demyelinating disease of the central nervous system. The pathogenesis of MS involves many processes, including cell survival, proliferation and immunomodulation. The TAM family of receptor tyrosine kinases (Tyro3, Axl and Mertk) plays a central role in these processes. We have previously identified 12 single nucleotide polymorphisms (SNPs) within the Mertk gene that are associated with MS susceptibility. We therefore investigated whether this polymorphism altered the expression of the Mertk gene. Methods: Using an Affymetrix Human Exon Array, we assessed Mertk expression in 25 treatment-naive patients with relapsing-remitting MS, as well as 24 controls in purified monocytes. Genotype at one of the identified Mertk MS-associated SNPs (rs17174870) was also determined in MS cases and controls. Results: This analysis established a significant correlated between genotype at rs17174870 and level of Mertk expression, with higher expression in all subjects carrying the risk allele ($p=5.56 \times 10^{-5}$). In a separate study of CD3+ T cells, we found a trend towards increased expression of Mertk (p=0.06) in MS patients compared with matched controls. Conclusions: In human monocytes, there is a strong correlation between the expression of the Mertk gene, and genotype at a SNP known to be associated with MS susceptibility, potentially linking levels of expression of this gene with disease pathology. We are currently extending these studies to other cell types, including B-cells and CD4+ and CD8+ T cells.

POS-TUE-101

EXPRESSION OF ACID SENSITIVE ION CHANNELS (ASIC) IN THE HUMAN COLON

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Purpose: Several classes of acid-sensitive ion channels (ASIC) have been identified in mammalian sensory neurons and may be implicated in different sensory modalities. To date, four mammalian ASIC genes have been cloned: ACCN2 (encoding ASIC1 subunits), ACCN1 (ASIC2), ACCN3 (ASIC3) and ACCN4 (ASIC4), which include spliced variants. ASICs exhibit different ranges of pH sensitivity, activation/ desensitisation kinetics, ion selectivity, tissue distribution and pharmacological properties. Some studies have been carried out in animal gut but there is almost no information about ASICs in the human intestine. Our aim was to examine expression of ASICs in the human colonic mucosa and muscularis. **Methods:** Human colon specimens from patients (26-77 years, n=24) were collected into RNA Later for molecular studies or fixed in Zamboni's for later immunohistochemistry. Immunostaining was carried out using a previously validated polyclonal ASIC1 antibody (Alomone Labs), using rat brain as positive control. **Results:** Expression of mRNA encoding for ASIC1, ASIC2, ASIC3 and ASIC4 protein was seen in both mucosa and muscle. Quantification using real time PCR showed no significant difference in expression between normal, ulcerative colitis (UC) and Crohn's disease (CD). Weak to moderate ASIC1-like immunoreactivity was observed on submucosal and myenteric ganglia, colonic smooth muscle, submucosal blood vessels and the mucosal epithelium of normal colon. In CD, preliminary studies appear to show an upregulation of ASIC1 immunoreactivity in mucosal epithelium and in submucosal ganglia and a decreased expression in colonic and vascular smooth muscle, compared with age-matched controls. **Conclusion:** ASICs are expressed in human colon, but further studies are required to evaluate their role in nociception and inflammatory bowel disease.

POS-TUE-102

NEUROCHEMICAL CHARACTERISTICS OF DORSAL ROOT GANGLION NEURONS IN C57/BL6 AND 129/J MICE IN RESPONSE TO NERVE INJURY

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Purpose & Methods: The mouse strains C57/Bl6 and 129/J differ in their behavioural response to nerve damage. We used multiple labelling immunohistochemistry to neurochemically characterize dorsal root ganglion (DRG) neurons 6 days after peripheral nerve damage (sciatic nerve transection). Nociceptive and non-nociceptive neurons were labelled with the markers Calcitonin Gene Related Peptide (CGRP), Isolectin-B4 (I-B4) and Neurofilament 200 (Nf200). The transcription factor ATF-3 and Neuropeptide Y (NPY) were used as markers of nerve damage. Size and 23 different neurochemical characteristics were determined sections of lumbar L4 DRG per animal. **Results:** Sciatic nerve transection induced in both strains expression of NPY and translocation of ATF-3 into the nucleus (129/J, n = 12 sections/2 animals, 1986 cells, C57Bl6, n = 12 sections/2 animals, 2706 cells). NPY (36±±1%, n=4) was exclusively expressed in ATF-3 positive neurons (80 % of ipsilateral neurons) and mainly in combination with Nf200-IR (33±11%, n=4). Nerve injury also reduced the number of I-B4 positive cells in DRG of both strains. Interestingly the average size of IB-4 positive neurons increased in response to nerve damage in both strains (contralateral 254±20µm, ipsilateral 300.6±20µm, n=4). Analysis of the neurochemical subpopulations showed that changes in response to nerve damage were similar between strains except that injury reduced the number of Nf200/ CGRP positive neurons in C57/Bl6 DRG but increased it in 129/J mice (15.4% to 6.3% vs 8.95% to 10%, n = 6 sections/2 animals condition). **Conclusion:** Changes in the neurochemical profile in response to nerve damage were mainly similar in nociceptive & non-nociceptive DRG neurones. The behavioural differences may be also dependent on differences in higher levels of the pain signalling pathway.

POS-TUE-103

EPHA4-FC ADMINISTRATION RESULTS IN FUNCTIONAL RECOVERY IN A RAT MODEL OF MODERATE CONTUSIVE SPINAL CORD INJURY

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Purpose: To evaluate the therapeutic effectiveness of EphA4-Fc in contusive spinal cord injury (SCI). **Methods:** A moderate contusive injury (150kdyne) was applied to adults rats at spinal level T10. EphA4-Fc was first administered 2 hours after injury at doses of 2 mg (n=11), 10 mg (n=12) or 40 mg (n=11) per kilogram bodyweight. Injured control animals received saline (n=12). Treatment continued at half the original dose administered every other day for 14 days. Behavioural assessment included the BBB locomotor score, the tapered balance beam task and sensory assays. The injured cords were examined using high-resolution MRI and DTI. **Results:** Rats treated with 2mg or 10 mg EphA4-Fc showed improved weight recovery with significantly higher body weight 76 days after injury ($p<0.05$). Locomotor ability assessed using the BBB score was significantly higher in the 2 mg group ($p<0.05$). However, rats treated with 10 mg or 40 mg of EphA4-Fc showed significantly greater ability compared to untreated rats on the tapered balance beam task ($p<0.05$). Although no differences were detected using DTI, high resolution MRI revealed less damage in the dorsal funiculus of rats treated with 10 mg or 40 mg EphA4-Fc ($p<0.05$). **Conclusion:** Therapy with EphA4-Fc after SCI enhances functional recovery and reduces damage to the spinal cord.

POS-TUE-104

IDENTIFICATION OF DEVELOPMENTALLY LINKED PROTEOMIC CHANGES IN THE SPINAL CORD OF *MONODELPHIS DOMESTICA*

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AIM: Physical trauma to the central nervous system (CNS) causes impairment of normal motor, sensory and autonomic functions. Using the laboratory opossum, *Monodelphis domestica*, this study aimed to identify proteomic changes in response to injury in the rostral segment of spinal cord (SC). Two stages of CNS development were used: postnatal (P) day 7 and P28 as previous studies showed that newborn pups injured in first two weeks of life recover morphologically and functionally but older pups do not (Wheaton *et. al.*, 2011). **METHODS:** SC injury was performed on newborn pups at either P7 or P28 at thoracic level (T) 10 under isoflurane anaesthesia. SC segment rostal to a complete transection was collected either 1 day or 7 days post-injury together with samples from age-matched control littermates. Proteomic profiling was performed by isoelectric separation followed by molecular weight separation on pooled samples (n=10 for P7-injured, n=4 for P28-injured). Gels were analysed by densitometry and protein bands that showed differences (±0.5) compared to controls, were identified. **RESULTS:** In total, SC injury affected 97 unique proteins at both ages. In P7-injured group, 53 proteins were affected of which 21 were up-regulated and 35 were down-regulated. In P28-injured group, 87 proteins were affected of which 44 were up-regulated and 43 were down-regulated. Mass spectrometry of identified proteins is in progress. **CONCLUSION:** We were able to identify proteins affected by SC which were developmentally linked. Ubiquitin, which responded to injury in the caudal segment of the SC (Noor *et. al.*, 2011) was also identified in the present study but its response was different.

POS-TUE-105

A SYSTEMATIC REVIEW OF LOCOMOTOR TRAINING AS A THERAPY IN ANIMAL MODELS OF SPINAL CORD INJURY

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Experiments on spinalized cats in the 1980s showed exercise could enhance functional recovery after spinal cord injury (SCI). **Purpose:** To systematically review evidence for the effectiveness of exercise training in promoting locomotor recovery in animal models of thoracic SCI. **Methods:** A systematic search of the literature was performed using Medline, Web of Science and Embase. Studies were included if they assessed any type of locomotor intervention in animals with SCI. A 13-item checklist was employed to assess the quality of included studies. **Results:** Of the 362 studies screened, 41 were included. Most studies (73%) showed exercise training had a positive effect on locomotor recovery. Studies employing a complete SCI were less likely to have positive outcomes. For incomplete SCI models, contusion was the most frequently used method of lesion induction, and the degree of recovery depended on injury severity. Positive outcomes were associated with training regimes that involved partial weight bearing, commenced within a critical period of one to two weeks after SCI, and maintained training for at least eight weeks. Considerable heterogeneity in training paradigms and methods used to assess recovery was observed. Only 15% of the studies had high methodological quality. Randomization of animals and blinding of assessments was often not reported. **Conclusions:** Exercise training following SCI improves recovery of locomotion in preclinical models of SCI. Future studies should be carefully designed to minimize sources of bias and would be improved if a small battery of objective assessment methods were developed and routinely employed across laboratories. This would allow future meta-analyses of the effectiveness of exercise interventions on locomotor recovery.

POS-TUE-106

DURATION OF TREADMILL TRAINING AND RECOVERY OF LOCOMOTION IN SPINAL CORD INJURED MICE

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Exercise is known to improve locomotion after spinal cord injury (SCI). However, there is little evidence regarding the length of training required to optimize recovery. **Purpose:** To compare the effects of treadmill training for 3 or 6 weeks on the recovery of locomotion after SCI. **Methods:** Adult male C57B1/6 mice received a spinal hemisection at T12. Prior to surgery animals were familiarized with running on a treadmill for 10 minutes, 2x per day, 5 days per week. One week after SCI, animals were randomized to trained (treadmill training for 3 or 6 weeks) or untrained groups. Kinematic gait analysis was performed at baseline, 1, 4 and 7 weeks after surgery using a high-speed camera to record step cycles of each animal. Spatiotemporal characteristics of locomotion and excursion of hindlimb joints were analyzed with the Peak Motus software. **Results:** Prior to SCI, all animals showed a coordinated pattern of locomotion. Following injury, the left hindlimb was paralyzed and the animals dragged their foot. Untrained animals (n=7) presented an uncoordinated locomotion that was maintained throughout the experiment. After 3 weeks of training, trained animals (n= 6) had an uncoordinated locomotor pattern and limited excursion of all hindlimb joints. After 6 weeks of training, there was recovery of hip and knee joints excursions similar to pre-injury values, but not at the ankle joint. **Conclusions:** Six weeks of treadmill training were show to improve locomotor pattern in SCI mice in comparison to no training or training for 3 weeks. A longer period of training might be necessary to improve excursions at all joints.

POS-TUE-107

EFFECT OF TREADMILL EXERCISE ON INTRINSIC AND SYNAPTIC PROPERTIES OF SPINAL NEURONS FOLLOWING SPINAL CORD INJURY IN ADULT MICE

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Exercise is known to promote functional recovery after spinal cord injury (SCI). However, the mechanisms mediating such improvements are not known. **Purpose**: To study the effect of exercise training after SCI on intrinsic and synaptic properties of spinal neurons. **Method**: Mice (C57Bl/6; 14 males, ~P42) received a spinal hemisection (T9-11) under isoflurane anesthesia. Following one week of recovery, animals were divided into exercise (treadmill exercise for 3 weeks) and non-exercise (control) groups. After 3 weeks, mice were sacrificed (decapitation, 100mg/kg, i.p. Ketamine). Horizontal spinal cord slices (T6-S1, 250μM) were prepared for whole cell patch clamp recording (23°C) in neurons in the vicinity of the SCI. **Results**: Neuronal input resistance (813 ± 165 vs. 697 ± 95MOhms), resting membrane potential (-62 ± 2 vs. -63 ± 2mV), action potential (AP) threshold (-40 ± 1 vs. -42 ± 1mV), and AHP amplitude (43 ± 2 vs. 47 ± 1mV) were similar in exercise (n = 20 neurons) and control (n =32) groups. Neurons were grouped into four AP discharge categories according to their response to current injection. The proportions of tonic firing, initial bursting, single spiking and delayed firing neurons was similar between groups. The frequency of spontaneous excitatory synaptic currents was also comparable (3.3 ± 0.8 vs. 2.4 ± 0.5Hz) in exercise (n = 22 neurons) and control (n =37) groups. **Conclusion**: These data show that 3 weeks of treadmill exercise after SCI has no effect on the selected intrinsic and synaptic properties of spinal neurons.

POS-TUE-108

CHARACTERIZATION OF A NOVEL COVALENTLY CROSS-LINKED Aβ PEPTIDE DIMER AND ITS ROLE IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by progressive memory loss, confusion, and cognitive deficits. While the major cause in AD is unknown, it is generally accepted that the beta amyloid peptide (Aβ) is toxic and we are confident that the toxic species are a low molecular weight soluble oligomeric species of Aβ that is responsible for neuronal dysfunction and synaptic loss. Furthermore, we propose that the formation of the toxic oligomeric species occurs via dityrosine crosslinking of the Aβ peptide (dY-Aβ), the process which is brought about by an oxidative modification reaction. It is a fundamental question in understanding Aβ neurotoxicity to test a bona fida dimeric Aβ with no monomer present. The complexity of synthesizing dityrosine-containing dimeric Aβ peptides has hampered research to directly test its toxic effects. The task of synthesizing an 84 amino acid peptide is a very difficult process and requires novel chemistry paradigms and experience in handling the Aβ peptide. We have developed novel methods for successfully synthesising the dY-Aβ dimer peptides containing up to 84 residues with high yields of pure peptides. To date, we have successfully synthesized and purified a number of Aβ monomers and respective dY-Aβ dimers and control DAP-Aβ dimers (this is the control dimer peptide using the 2,6-diaminopimelic acid in place of dityrosine during the synthesis of the Aβ dimer). Our results show that the synthetic Dimer peptide is more toxic than the monomer equivalent peptide. This is associated with increased cell binding to neurons. These findings suggest that the synthetic dimer peptide may be a better peptide model for investigating Aβ neurotoxicity associated with AD.

POS-TUE-109

HETEROGENEOUS RESPONSE TO MECHANICAL STIMULI IN ISOLATED VAGAL TRACHEAL MECHANOSENSORY NEURONS

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Purpose: The cough reflex protects the airways and lungs from potentially damaging stimuli. Recent studies have described a novel airway mechanosensitive (MS) neuron in guinea pigs that is critical for initiating defensive coughing, although very little is known about the mechanical transduction mechanisms in MS neurons. Mechanical stimuli applied to the epithelial surface of the trachea evoke high frequency firing in airway MS neurons. These neurons have been classified as rapidly adapting, based in their lack of sustained action potential generation during a maintained mechanical stimuli applied to their terminal fields. However, it is unclear whether this adaptation reflects the electrophysiological properties of the MS neurons or time-dependent changes that may occur in the viscoelastic properties of the surrounding tissue to which the stimuli are applied. **Methods:** We have performed patch-clamp recordings of isolated retrogradely traced tracheal nodose neurons to investigate the mechanical activation and adaptation properties by directly stimulating the membrane of the neurons. **Results:** 100msec mechanical stimulation of nodose neurons evoked MS inward currents. Based on the relaxation time constant, three kinetically distinct MS currents were distinguished; rapid (70% of neurons), slow (15% of neurons), and ultra-slow (15% of neurons). Whole cell capacitance, and time to peak responses differed significantly ($P<0.05$) between rapid and slow neurons. Repeated stimulations of short (100ms) or long (1000ms) duration produced adaptation/desensitization of both fast and slow MS currents, although the loss of responsiveness in slow neurons to longer stimuli was more pronounced than in rapid neurons. **Conclusion:** These data suggest that multiple types of tracheal MS neurons exist based on MS adaptation properties. The role of these subtypes in reflex evoked coughing in response to mechanical stimuli is at present unclear.

POS-TUE-110

DEVELOPMENT OF THE FOVEA AND VISUAL RESOLUTION IN THE TROPICAL SEAHORSE (HIPPOCAMPUS TAENIOPTERUS)

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Purpose: Seahorses are visually guided feeders requiring high resolution vision to prey upon small, fast moving crustaceans. They possess a convexiclivate fovea characterised by a rod-free depression. In this study, we analysed the developmental changes in foveal morphology, cell density and Reactive distance, a behavioural measure of visual resolution. **Methods:** Three age groups of tropical *H. taeniopterus* seahorses were analysed (Group1- juvenile fish (6 cm; n=6), Group2 (10 cm; n=5) and Group3 - adults (15 cm in length; n=10). Following Reactive distance measurements, eyes were processed for frozen sections or flat-mounted. Wholemounts were stained with propidium iodide and immunolabeled with Ret-P1, a rod opsin marker. Photoreceptor and ganglion cell densities were determined and topography maps constructed. **Results:** The bigger fish were able to detect the same sized prey at a greater distance. Morphologically, the depression of the pit appears larger as the fish grows. The area of rod-free zone increased in size with age. The average peak photoreceptor cell density increased during development from 221,200 cells/mm^2 (Group1) to 298,250 cells/mm^2 (Group3), whereas the average peak ganglion cell densities decreased from 69,800 cells/mm^2 to 50,000 cells/mm^2. Both the theoretical and the behavioural limits of visual resolution decreased with age. **Conclusions:** *H. taeniopterus* fovea development is similar to that of other foveate teleost retinas in that there is a decrease in ganglion cell densities with age. Total photoreceptor densities increase as the fish grows. This change in morphology is correlated with an increased visual function.

POS-TUE-111

EARLY MOLECULAR CHANGES IN HUNTINGTON'S DISEASE TRANSGENIC MICE ASSOCIATED WITH AFFECTIVE AND COGNITIVE DEFICITS AND THE MODULATORY EFFECTS OF ENVIRONMENTAL STIMULI

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Huntington's disease (HD) is a fatal tandem repeat (CAG) expansion disorder involving a triad of psychiatric, cognitive and motor symptoms. Purpose: In a transgenic mouse model of HD (R6/1 line) we have characterized onset of affective and cognitive endophenotypes, which precede motor deficits. We have also demonstrated that environmental enrichment can delay onset of these endophenotypes in HD mice. The molecular mechanisms mediating HD pathogenesis, and their modulation by environmental stimuli, have been investigated in the present study. Methods: We performed a microarray experiment on neocortex, hippocampus and striatum of R6/1 HD mice, and validated the results using quantitative real-time PCR and other approaches. We also tested the hypothesis that environmental enrichment (EE) and wheel running (RW) improve behavioural performance through changing gene expression patterns. Results: The microarray experiment demonstrated early gene expression changes are occurring in multiple brain regions of R6/1 HD mice. We found that even at the early pre-motor symptomatic age of 8 weeks, gene expression was dysregulated, with regional and sex-specific differences apparent, including specific serotonin (5-HT) receptors. The gene level changes were reflected in the receptor binding results, with 5-HT2a, 5-HT1a and 5-HTT binding all decreased in at least one brain region at 12 weeks, demonstrating that transcriptional changes affect protein levels. Conclusions: These findings suggest that disruption of specific neuromodulatory systems occurs early in particular brain regions of HD mice, and may be involved in the affective and cognitive endophenotypes we have described. Our results also suggest that the timing and duration of environmental manipulations are critical in terms of their ability to modify gene expression.

POS-TUE-112

MIGRAINE- ONWARD AND DOWNWARD

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Purpose. How migraine pain arises is a mystery. It has been attributed to a pathology of the cranial vasculature and dura mater, but no such pathology has ever been found- we cannot test for migraine. We posit that migraine pain arises from changes in cortical excitability, which lead to a withdrawal of descending inhibitory control by the brainstem nuclei. This leads to the misperception of "native" traffic as a pain state. Methods. We conducted neurophysiological experiments in 230 rats using a variety of techniques, accepting $P<05$ as our criterion for significance. Results. (1) Migraine triggers (cortical spreading depression and light flash): (a) decrease the drive of neurons in the periaqueductal gray matter (PAG) and the nucleus raphe magnus (NRM); (b) increase the drive of trigeminovascular second-order sensory neurons and; (c) increase the responsiveness of second-order trigeminovascular sensory neurons to noxious stimulation of the dura mater. (2) These effects can be antagonised by brainstem injection of the neuronal excitant glutamate and potentiated by brainstem injection of lignocaine, a local anaesthetic into the PAG or NRM. (3) Migraine triggers do not increase traffic in peripheral sensory fibres of the trigeminovascular system. (4) Electrical stimulation of the PAG or NRM suppresses responses of trigeminovascular second-order neurons to stimulation of the dura mater. (5) Somatostatin is an important neurotransmitter involved in controlling the drive of the PAG and NRM from the cortex. (6) Nearly all of these effects are exclusive to the trigeminovascular sensory system and are NOT paralleled by similar effects on other trigeminal sensory systems. Conclusions. These findings go some way towards uncovering the cause of migraine pain and towards explaining some of its characteristic features, especially gender differences and hormonal influences.

POS-TUE-113

TEMPORAL SEQUENCE OF TISSUE DAMAGE AFTER SPINAL CORD INJURY

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Purpose: Traumatic spinal cord injuries are devastating for patients because they often result in sudden and premanent loss of motor, sensory and autonomic functions below the level of the injury. The extent of functional disability suffered is determined by the extent of tissue damage and the spinal level at which the injury occurs. Tissue damage is known to occur in 2 main phases, initial impact damage at the time of injury followed by a period of secondary damage during which more tissue is gradually lost. Because this is tissue that had survived the initial impact, treatments to limit the extent of secondary damage have the potential to limit the extent of functional disability ultimately suffered. However, these treatments would need to be administered before this tissue has become irreversibly damaged. Thus it is important to determine the timing of tissue loss in order to define the theraputic window available for intervention. **Methods:** Anaesthetised adult rats were subjected to controlled lower thoracic spinal cord contusion injuries and the extent of tissue damage assessed in serial sections spanning the entire injury site at different times after injury (n=4-5 per time point). **Results:** Damage to spinal grey matter is rapid and complete by 24 hours post-injury. In contrast, damage to white matter occurs over a longer period. By 24 hours post-injury, 50% of myelinated white matter axons had been lost at the lesion centre, rising to 95% by 1 week post-injury. No further significant loss of myelinated axons occurred after 1 week post-injury. **Conclusion:** Potential neuro-protective treatments would need to be administered within hours of injury if they are to have any chance of limiting tissue damage.

POS-TUE-114

SITE-SPECIFIC EXCITOTOXIN EXPOSURE IN VIVO LEADS TO NEURONAL EXCITOTOXICITY AND AXONAL DYSFUNCTION

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Purpose: ALS is likely to be a multifactorial disease of neuronal dysfunction and loss, however, recent investigations indicate that axonal dysfunction, prior to cell loss, may be the causative factor of the initial symptoms of ALS and that distal axonal degeneration may occur before the onset of disease symptoms. Our investigations are focused on determining the degenerative changes underlying ALS-like axonopathy by using site-specific excitotoxic insults, via osmotic minipumps (Alzet, model 1004), *in vivo*. **Methods:** A constant and chronic infusion of Kanic acid (KA, 1-5mM, in cortex buffer with 2μM Fluro Ruby) was delivered to the subarachnoid space of the lumbar region (L3-5) of C57/Bl6 mice and transgenic mice which express yellow fluorescent protein (YFP) in a subset of motor neurons on a C57/BL6 background. **Results:** Fluoro Ruby labelling was present throughout cells within the subarachnoid space in L3-5) and a small number of neurons within the ventral horn, indicating a targeted delivery can be achieved with the osmotic pumps. By 28 days post surgery (DPI) there was a significant ($p<0.05$) decrease in the number of SMI-32 positive neurons in the anterior ventral horn of 5mM KA treated mice. Additionally there was a significant ($p<0.05$) reduction in the number of double-labelled synaptophysin and alpha bungarotoxin synapses in the gastrocnemius muscle of the 5mM KA treated mice. Kainic acid treated YFP mice also demonstrated neuromuscular junction retraction and degeneration by 28 DPS, however this degeneration had occured as early as 14 DPS. **Conclusion:** Identifying the site of the initial effects of excitotoxicity will identify mechanisms of distal axon degeneration that may provide novel therapeutic targets directed at axon protection.

POS-WED-001

ADOLESCENT TESTOSTERONE AND POSTNATAL NEUROGENESIS IN THE PRIMATE HIPPOCAMPUS

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Purpose: New neurons are continuously produced in the dentate gyrus (DG) and CA4 region of the adult hippocampus and reduced cell proliferation is found in schizophrenia in these regions. Evidence from non-primates indicates that sex steroids can modulate adult hippocampal neurogenesis. While onset of schizophrenia symptoms often occurs in adolescence, little is known of the effect of sex steroids on neurogenesis during primate adolescence. This study aims to determine the extent to which adolescent testosterone changes levels of cell proliferation in the rhesus macaque hippocampus. **Methods:** Prepubertal male rhesus macaques were gonadectomised (n=6) or sham-operated (n=6) at 2.5 years of age then sacrificed at 4.5 years of age. Brains were sectioned coronally along the dentate gyrus (–9.90 to -21.25mm bregma). Immunohistochemistry was performed in 5-6 sections per brain to detect the cell proliferation marker Ki67 in the granule cell layer and subgranular zone of the DG, and in CA4. Stereological methods were used to estimate the volume of the total hippocampus, DG and CA4. **Results:** We did not detect a significant difference in the volume of the hippocampus between gonadectomised and intact animals. Our results indicate in intact animals an increase in the total number of Ki67+ cells in CA4 (~40%) and an increase in the total number of Ki67+ cells in the DG (~25%). **Conclusion:** The presence of circulating testosterone may increase cell proliferation in the primate hippocampus during adolescence. Future studies are needed to determine if the number of immature and/or surviving neurons is similarly changed in the presence of testosterone.

POS-WED-002

NO CHANGE IN PROGENITOR CELL PROLIFERATION IN THE HIPPOCAMPUS IN HUNTINGTON'S DISEASE

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Purpose: In the subventricular zone (SVZ), animal models of Huntington's disease (HD) have demonstrated no change in cell proliferation compared to wild types, while in humans there is a distinct increase in cell proliferation in HD cases. However, no previous study reports on cell proliferation in the human subgranular zone (SGZ) of the hippocampus in HD, despite numerous transgenic mouse models of HD showing decreased proliferation in the SGZ. **Methods:** In this study we examined hippocampal proliferation in HD (n=14) and normal (n=8) brains. HD cases were analysed as a whole group and according to symptom profile: mood, motor or mixed symptoms, to determine whether HD and symptom profile, had an effect on SGZ proliferation. We used a range of cell-cycle protein markers including; proliferating cell nuclear antigen, mini chromosome marker 2, and B-cell lymphoma 2 protein, to label proliferating cells in the SGZ. The length and surface area was also measured and used to calculate proliferating cells per mm, and per μm^2. **Results:** Overall, the results showed minimal proliferation in the adult SGZ, which is comparable with previous studies on the human hippocampus. Most importantly, our results reveal no significant differences in SGZ proliferation between normal and HD symptom profiles. **Conclusion:** These results demonstrate that in humans the SGZ is far less proliferative than the SVZ, and suggests that hippocampal plasticity in humans does not primarily involve cell proliferation.

POS-WED-003

DO MULTIPOTENT NEURAL PROGENITOR CELLS REMAIN IN THE MATURING PRIMATE NEOCORTEX?

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Purpose: To identify in vitro self-renewing multipotent neural progenitor cells (NPCs) derived from the postnatal nonhuman primate primary visual cortex (V1) at postnatal day 14. **Methods:** The presence of proliferative progenitor cells was first assessed *in vivo* by co-labelling with specific markers. The *in vitro* analysis was performed using the neurosphere assay (n=11): V1 cells at PD14 were dissociated and cultured in presence of epidermal growth factor (EGF) and/or fibroblast growth factor-2 (FGF-2). The differentiation into neurons, oligodendrocytes and astrocytes was assessed by immunolabelling. **Results:** While neuronal maturation within V1 neocortex is well advanced by PD14, we observed cells co-expressing Sox2 and Ki67 throughout the cortical layers, defining a population of resident proliferating progenitor cells. When cultured in neurosphere conditions, we observed the formation of neurospheres containing mitotically active $Sox2^+$ progenitor cells. When dissociated into single cells, neurospheres could be generated for over 6 passages. Dissociated neurospheres cultured in presence of serum differentiated mainly into $GFAP^+$ astrocytes, however a small but consistent proportion of $MAP2^+$ neurons and $O4^+$ oligodendrocytes could be observed for over 3 passages. After the third passage, we only observed astrocytes. **Conclusion:** This study provides the first direct evidence for the existence of multipotent NPCs within the postnatal neocortex of the nonhuman primate. As it is often observed with late progenitors, the cells identified here have a limited multipotentiality. Our results also revealed that FGF-2 is more potent than EGF in supporting the formation and growth of the neurospheres. The potential contribution of neocortical NPCs to neural repair following injury raises exciting new possibilities for the field of regenerative medicine.

POS-WED-004

SPECIFIC UPREGULATION OF NEUROGENESIS IN THE SUBSTANTIA NIGRA PARS COMPACTA IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Purpose: We previously reported that significant neurogenesis occurs spontaneously in the adult mouse midbrain, from Nestin+ neural precursor cells (NPCs). The present experiments were to determine if midbrain neurogenesis is upregulated in response to specific depletion of substantia nigra pars compacta (SNc) dopamine (DA) neurons, whether NPCs differentiate into glutamate neurons, and to what extent these NPCs incorporate Bromodeoxyuridine (BrdU). **Methods:** We used adult (≥8 weeks old) transgenic mice in which transient Nestin expression permanently drives β-galactosidase (LacZ) expression (in the presence of tamoxifen). One group of mice (n=10) received a unilateral 6-OHDA (or sham) injection and were perfused 8 weeks later. A second group of mice (n=6) received BrdU (50mg/kg i.p. twice/day) for 4 days and were perfused 2 weeks later. Brain sections were immunohistochemically processed to visualize LacZ, BrdU, and markers of astrocytes and neurons (including glutamate and DA). **Results:** (1) Following 6-OHDA the number of LacZ+ cells in SNc, and LacZ+/NeuN+ cells in the periaqueductal gray were significantly ($p \leq 0.05$) increased. (2) No midbrain LacZ+ cells were glutamatergic. (3) Similar densities [cells/10000μm^2] of LacZ+ [5.4] and BrdU+ [6.8] cells were present in the olfactory bulb, but few [1] were LacZ+/BrdU+. In midbrain there were many more LacZ+ [7.4] than BrdU+ [1.125] cells, and also few LacZ+/BrdU+ cells [0.67]. There were no LacZ+/BrdU+ cells in SNc. **Conclusions:** Midbrain neurogenesis is upregulated in response to SNc DA cell depletion and new cells are directed to SNc. New midbrain neurons are not glutamatergic. BrdU labels a largely different population of newborn cells to Nestin.

POS-WED-005

LONG TERM MAINTENANCE DIRECTS ES CELL-DERIVED NEURAL STEM CELLS TOWARDS A FOREBRAIN GABAERGIC PHENOTYPE

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Background: Neural stem (NS) cells are defined by their capacity to proliferate and differentiate into neuronal and glial phenotypes; they are predominantly found in specific regions of the adult brain, or they can be generated from embryonic stem (ES) cells. When cultured in EGF and FGF2, ES cell-derived NS cells have been reported to be stable and multipotent. However, conditions that enable differentiation of NS cells through the committed progenitor and precursor stages to specific neuronal subtypes have not been fully established. Previously, we have shown that extended periods of neural induction enhanced the potential of mouse ES cell-derived NS/NP cells to generate specific subtypes of neurons. **Purpose:** In this study we now investigate, using Lmx1a reporter ES cell lines, the effect of EGF and FGF2 (EGF/FGF2) on the neurogenic potential of NS/NP cells over extended periods of cultivation. Methods: Following monolayer neural induction, neurosphere formation and subsequent replating, the NS/NP cells were propagated for up to 10 passages in the EGF/FGF2. The NS/NP cultures differentiated at passages 2, 5, 7 and 10 over 16 days and then used for terminal differentiation and subsequent immunolabelling. **Results:** Passage 2 NS/NP cells could be differentiated mainly toward a forebrain phenotype, evident in the comparatively high percentage of Special AT-rich sequence-binding protein 2 (SatB2; 11.9±3.1%; n=3) positive neurons; as well as catecholaminergic and GABAergic neurons, indicated by the presence tyrosine hydroxylase (TH; 13.2±1.6%; n=3), gamma-aminobutyric acid (GABA; 34.6±3.0%; n=3) of the βIII-tubulin positive (44.8±12.6%; n=3) population. Over the successive passages, the NS/NP cells displayed a bias towards the GABAergic phenotype with the loss of catecholaminergic potential by passage 10 (TH: 1.3±0.1%; GABA: 36.9±2.1%; n=3) while neurogenic potential is retained (βIII-tubulin: 50.0±8.1%; n=3). **Conclusion:** Our data suggests that the initial monolayer neural induction, followed by long term maintenance in the presence of EGF and FGF2 directs ES cell-derived NS/NP cells towards a forebrain GABAergic phenotype.

POS-WED-006

NEURONAL DIFFERENTIATION OF MURINE DENTAL PULP STEM CELLS *IN VITRO*

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Purpose: The potential of dental pulp stem cells (DPSCs) in regenerative medicine is becoming more widely understood. The ability of murine DPSCs to become chondrocytes, osteocytes and adipocytes has recently been demonstrated by several groups, but their neurogenic potential has not been shown. In this study we demonstrate neuronal differentiation of murine DPSCs *in vitro*. **Method:** mDPSCs were cultured *in vitro* under neuroinductive conditions (n=12) for 14 days. Cultures were subsequently analysed for neuronal and synaptic markers by immunohistochemistry (IHC) or whole cell patch clamped to assess intracellular electrophysiology. mDPSCs were also seeded onto microelectrode arrays (n=5) to assess network activity. **Results:** The mDPSCs showed a neuronal phenotype from 5 days in a neuroinductive media and developed a neuritic network of processors. IHC revealed approximately 20 percent of cells expressed the pan-neuronal marker β-III tubulin and approximately 40 percent GFAP. mDPSCs also expressed markers for NeuN, Connexin 43 and low levels of synapsin 1, periaxin and S100. Intracellular electrophysiological analysis revealed the presence of voltage-gated sodium channels in a subset of neuronal-like cells, which were TTX-sensitive. No potassium currents were found and the cells did not support spontaneous action potentials. The addition of a gap junction blocker reduced the capacitance of the networks significantly. Furthermore, lucifer yellow or neurobiotin injected intracellularly spread widely within cell clusters of neurons and glia, demonstrating the presence of gap junctions. **Conclusion:** These data suggest the differentiation of mDPSCs into a heterogeneous population of neural-like cell networks that could represent an early stage of neural development.

POS-WED-007

ABSENCE OF *RET* INCREASES CELL CYCLE LENGTH IN PROLIFERATING NEUROBLASTS IN THE DEVELOPING MOUSE STELLATE GANGLION

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Purpose: Sympathetic ganglia form from small numbers of neural crest cells, which then proliferate and differentiate into neurons and glia. The number of cells produced depends on both cell cycle length and changes in the growth fraction (the number of cells remaining in the cell cycle at any time). Deletion of the GDNF family ligand co-receptor gene *Ret*, results in reduced numbers of sympathetic neurons. We compared wild type to animals homozygous for deletion of *Ret*. **Methods:** We measured changes in cell cycle length and growth fraction in proliferating cells in mouse stellate ganglia using labelling with two S-phase markers and immunohistochemistry (IR) for Ki67 from embryonic day (E)9.5 to E18.5 (n= minimum 3 for each age). Neuroblasts were identified using immunohistochemistry to Phox2b or tyrosine hydroxylase, and neural crest cells and glial cells identified by immunohistochemistry to Sox10. **Results:** In wild type animals, the growth fraction was 1 until E12.5 for both neuroblasts and Sox10-IR cells. From E11.5 onwards cell-cycle length for neuroblasts was constant at around 20 h, while that of Sox10-IR cells tended to increase from 12 h to 38 h. Deletion of *Ret* resulted in an increase in the cell-cycle length of neuroblasts on E16.5 (to 125 h). Cell-cycle dynamics of Sox10-IR cells was not altered by the deletion of *Ret*. **Conclusion:** *Ret* is only expressed by significant numbers of sympathetic neuroblasts up until E13.5, suggesting either that the absence of *Ret* early in development has disrupted cell-cycle regulation at later times or the extra-ganglionic consequences of the absence of *Ret* (e.g. the absence of kidneys) impacts on sympathetic development.

POS-WED-008

DIRECTING DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO AUDITORY-LIKE NEURONS

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Purpose: Degeneration of auditory neurons (ANs) due to deafness may have a negative impact on patients' clinical performance with a cochlear implant. Therefore studies in our laboratory have focused on replacing ANs using human embryonic stem cells (hESCs). This project is unique in its aim to characterise the potential of human induced pluripotent stem cells (iPSCs), to be differentiated toward an AN lineage. **Methods:** Using our published in vitro assay, iPSCs and hESCs (control) were directed towards a sensory neural lineage. The expression of 6 sensory neural markers were analysed at 19, 21, 24, 28 and 35 days *in vitro* (DIV) from the start of the differentiation assay, using immunocytochemistry. **Results:** Both iPSC and hESC neurospheres expressed the dorsal marker Pax7 at 21 (n=8) and 24 DIV (n=9) and the cranial placode marker Pax2 at 19 (n=24), 21 (n=21) and 24 DIV (n=12). This was followed by expression of the sensory neural markers Brn3a at 24 (n=22), 28 (n=38), 35 DIV (n=10) and Islet1 at 35 DIV (n=10). The neural specific marker βIII tubulin was expressed at 19 (n=24), 21 (n=21), and 24 DIV (n=12) and Neurofilament 160 kDa at 24 (n=22) and 28 DIV (n=38). **Conclusions:** This study illustrates that iPSCs and hESCs have similar (but not identical) differentiation potentials using our *in vitro* assay. Additionally, the pattern of marker expression in iPSCs is consistent with the timeline of marker expression in hESCs. Future investigations using Q-PCR will more accurately determine the relative expression of these proteins in iPSCs and hESCs.

POS-WED-009

NEURAL DIFFERENTIATION OF PATIENT DERIVED OLFACTORY CELLS TO STUDY CELLULAR TRAFFICKING

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Purpose: Hereditary Spastic Paraplegia (HSP) is an inherited neurological disorder characterized by progressive lower limb spastic weakness and paralysis. It is attributed to retrograde degeneration of the nerve fibers in the upper motor neurons and the dorsal columns. The treatment for HSP is only symptomatic, aimed at reducing muscular spasticity. Patients with mutations in the SPG4 gene are the focus of our study as it is responsible for 40% of autosomal dominant adult-onset cases. Comparison of olfactory neurosphere-derived (hONS) cells from SPG4 HSP patients and healthy controls show significantly less stabilized tubulin in patient cells and altered distribution of peroxisomes and mitochondria. **Aim:** The goal of this project is to find a reliable protocol to differentiate HSP hONS cells into neurons and glia. The most efficient protocol will be used to study trafficking defects of mitochondria and peroxisomes. Trafficking studies with undifferentiated cells has been hampered as the cells constantly contract and retract. Differentiated cells that exhibit long extended processes will also better model the long corticospinal axons that appear to be most affected by HSP. This patient-derived model could lead to potential targets for drug screening. **Methodology:** Three protocols are used for neural-differentiation. Protocol 1, previously used for embryonic and induced pluripotent stem cells involves dual inhibition of the SMAD pathway with Noggin & SB431542. Protocol 2 involves growing hONScells in Neurobasal medium supplemented with B-27, Glutamine and Glutamate for 5-6 weeks. Protocol3 involves growing the cells in DMEM, ITS, EGF and FGF for 6 days **Results**: All the three protocols induced elongated morphology. At day 15 Protocol1 produced differentiated cells with long-extended processes positive for GFAP (>90%). From day 6-21 protocol 2 produced morphologically extended cells and these will be followed for 2 weeks and immunostained for neural markers. At day 6 Protocol3 produced cells with long extensions, these cells will also be immunostained for neural markers.

POS-WED-010

USING EMBRYONIC STEM CELLS TO PROVIDE HUMAN NEURONS FOR DRUG SCREENING

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Background: Over 500 candidate drugs are reported to provide neuroprotection against ischaemic injury in animal models of stroke but none have been shown to work in humans. Many explanations for this translational failure have been proposed but the simplest, that the drugs are not appropriately active in humans, has not been explored. The aims of this study were to differentiate human embryonic stem cell (hESC) lines into neurons and develop models of ischaemic injury to test candidate drugs in human cells. **Methods:** Human ESCs were differentiated into neurons in the presence of the bone morphogenic inhibitor protein, Noggin. The mature neurons were maintained for 11 days prior to the induction of injury. Two injury models were used, oxygen glucose deprivation and oxidative stress. Three candidate therapies (hypothermia, melatonin, and NXY-059) were tested for their effect on cell death quantified using a lactate dehydrogenase assay. **Results:** Hypothermia and Melatonin were neuroprotective after four hours of OGD injury and oxidative stress, reducing cell death by 28% and 33.6% (Hypothermia) and by 44.2% and 37.8% (100μM Melatonin) respectively compared to controls. However, NXY-059 had no effect on neuronal cell survival in either of the injury models. **Conclusion:** Human neurons derived from embryonic stem cells can be used for drug screening. Identifying neuroprotective agents that work in such human *in vitro* systems may bridge an important gap between animal studies and clinical trials.

POS-WED-011

THE CALCIUM SENSOR, STIM2 IS LOCALISED TO RADIAL GLIA WITHIN THE DEVELOPING NERVOUS SYSTEM

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Correct function in the adult central nervous system (CNS) is dependent on the highly regulated processes of neurodevelopment. Vital to neuronal development is the generation of appropriate calcium signals within the developing neuronal-glial network. **Purpose**: Our work focused on the calcium regulatory protein, Stromal Interaction Molecule 2 (STIM2) in the developing nervous sytem. STIM2 is located on the surface of the endoplasmic reticulum and triggers a restorative calcium influx in response to calcium depletion[1]. STIM2 has been described as the neuronal isoform of the STIM proteins[2]. We hypothesise that STIM2 is developmentally regulated in neurons over embryonic maturation. **Methods**: STIM2 expression was quantified by Western analysis in the CNS of rats aged embryonic day 15 (E15), E17 and E19 (n=3 for each age). **Results**: There was no significant difference observed over this developmental period in the brain (E15: 1.63±0.86, E17: 1.47±0.85, E19: 2.71±2.91 p>0.05), or spinal cord (E15: 1.03±0.60, E17: 0.74±0.25, E19: 1.24±0.39, p>0.05). We found that STIM2 was not developmentally regulated and furthermore, it was not localised to neurons. Characterisation of STIM2-immunoreactivity showed that STIM2 was localised to long cellular processes that spanned from the ventricular zone to the pial surface. Co-localisation with nestin indicated that STIM2-was localised exclusively to radial glial cells within the embryonic brain and spinal cord. **Conclusions**: We hypothesise that STIM2 plays a role in the maintenance of radial glial calcium waves, thereby functioning as an important mediator of neurogenesis and structural organisation of the mature CNS. 1: Brandman, O. *et al*., 2007. Cell, 131(7), pp.1327–1339. 2: Berna-Erro, A. *et al*., 2009. Science Signaling, 2(93), p.ra67.

POS-WED-012

NFIX REGULATES THE DIFFERENTIATION OF NEURAL STEM CELLS THROUGH THE REPRESSION OF SOX9

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Neural stem cells (NSCs) are self-renewing cells that have the ability to give rise to neurons and glia in the embryonic, neonatal and adult brain. The control of NSCs formation and maintenance is essential for the proper development of the central nervous system. Sox9 is a member of the SoxE family and it plays an important role in the formation and maintenance of NSCs. However, it is still unclear on how Sox9 expression is regulated in the developing cortex. Here we demonstrate that *Nfix* plays a role in the repression of Sox9 expression. During CNS development, *Nfix* is expressed by NSCs in the hippocampus, and also *Nfix*$^{-/-}$ mice also display a significant increase in Sox9 expression as shown by qPCR and immunohistochemistry. Furthermore reporter gene assays demonstrated that *Nfix* was able to directly repress luciferase expression driven by the Sox9 promoter in vitro. Collectively, these data suggest that *Nfix* drives the differentiation of NSCs through the repression of Sox9.

POS-WED-013

EARLY ENVIRONMENTAL ENRICHMENT INFLUENCES THE RATE OF PARVALBUMIN-POSITIVE INHIBITORY INTERNEURON DEVELOPMENT WITHIN THE STRIATUM

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Purpose: The critical period is the time of peak plasticity within the nervous system. Parvalbumin positive (PV+) inhibitory interneurons are thought to play a vital role in regulating the timing of this important developmental epoch by consolidating circuits formed by maturing excitatory neurons. This study investigates whether environmental enrichment from birth can specifically influence the morphological development of PV+ neurons within the striatum. **Methods:** C57BL6J mice were raised in enriched (EE) or standard environments (SE) from birth. Brains were taken at P10, P15, P21 and adulthood (n=4). Immunohistochemistry against PV+ neurons was conducted, and images taken using fluorescent confocal microscopy. Morphological parameters including number of cells, soma size, dendritic surface area and volume were measured for PV+ cells within rostral, middle and caudal striatum. A repeated measures ANOVA (environmental condition as between subject factor) was used to compare values. **Result:** As animals matured there was a significant interaction between environmental condition, age, and rostral-caudal level of the striatum. These factors affected soma size (repeated measures (rm) ANOVA, $p<0.001$) and also number of cells (rmANOVA, $p<0.05$), suggesting PV+ inhibitory interneurons in the striatum of EE animals undergo accelerated maturation. **Conclusion:** These findings indicate that environmental conditions can have a complex and dynamic effect on the maturation of PV+ neurons throughout the rostral-caudal span of the striatum. The fact that this particular cell type is influenced by changes in the animals' surroundings suggests a further means by which experience can impact the timing of developmental critical periods.

POS-WED-014

CALCIUM SIGNALLING IN ADULT RADIAL GLIA-LIKE PRECURSOR CELLS

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Adult neurogenesis is well established in the subventricular zone (SVZ) lining the lateral ventricles of the adult mammalian brain. Neural precursor cells of this neurogeneic niche continuously produce neurons that mature and become integrated into functional networks that are involved in odor discrimination in the olfactory bulb. Neural precursor cells can be harvested from the SVZ and cultured as heterogeneous neural aggregates referred to as neuropsheres. Adult SVZ derived neurospheres are thought to contain multipotent neural stem cells and when plated in vitro were observed to generate cells of the three main neural lineages: βIIItubulin+ neurons (0.012 ± 0.057%); $GFAP^+$/$vimentin^-$ astrocytes (0.033 ± 0.017%); and $O4^+$ oligodendrocyte precursor cells (0.091 ± 0.13%). Calcium signalling was investigated in two neural phenotypes observed to emerge from neurospheres and identified as $vimentin^+$/$SOX2^+$/DCX^-/$GFAP^-$ radial glia-like (B type) cells and DCX^+/$SOX2^+$/$vimentin^-$/$GFAP^-$ neuronal precursor (A type) cells. Responses to the purinergic agonist ATP (50μM) and growth factor mitogens EGF (10ng/ml) and bFGF (10ng/ml) were examined and their downstream signalling cascades analysed. Both B type and A type cells were found to be responsive to depolarisation and exhibited mechanisms of SOC channel activation. This study focuses primarily on the spontaneous calcium oscillations observed in radial glia-like cells. Through the use of the antagonists ruthenium red (10μM), ryanodine (100μM), 2-APB (5μM), xestospongin-C (1μM), U-73122 (5μM) and thapsigargin (1μM) the spontaneous calcium oscillations were found to be regulated at the IP_3R on the ER. Taken as a whole, these results offer novel insight into the calcium physiology of SVZ-derived neural precursor cells.

POS-WED-015

THE ROLE OF LMX1A DURING *IN VITRO* MONOLAYER NEURAL DIFFERENTIATION

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Background Cell replacement therapies offer some level of hope in the treatment of incurable neurodegenerative conditions, such as Parkinson's disease. The main drawback with this type of replacement strategy is the derivation of appropriate specific neuronal phenotypes in sufficient quantities from stem cell cultures. Lmx1a is a transcription factor that plays a central role in the in vivo development of dopaminergic neurons. **Purpose** In this study we investigate the development of dopaminergic neurons and attempt to isolate commited dopaminergic progenitors derived from embryonic stem (ES) cells. **Methods** We performed fluorescent activated cell sorting (FACS), qPCR and immunocytochemistry on a Lmx1a-Amp-IRES-GFP knock-in reporter cell line. **Results** During monolayer neural differentiation, the number of Lmx1a-Amp-IRES-GFP positive cells (n=3) in culture peaks at day 8. This expression was reduced by the addition of BMP and Wnt (n=3). However, inhibition of Wnt or BMP increased tyrosine hydroxylase (TH) expression (n=3) at day 20. Sonic hedgehog (Shh) did not affect Lmx1a expression or TH positive cell numbers (n=3). qPCR on FACS isolated Lmx1a-Amp-IRES-GFP positive and negative cells (both n=3) showed that genes associated with dopaminergic neuron differentiation (Msx1, Wnt1, and Lmx1b) were upregulated in positive cells. When replated and allowed to differentiate, isolated positive cells (n=3) predominantly gave rise to GABA positive neurons. **Conclusion** Monolayer culture derived Lmx1a cells do not reflect the development of dopaminergic neurons but are instead, under our monolayer culture conditions, committed towards a GABAergic or glutamatergic neuronal fate.

POS-WED-016

MARGINAL ZONE CELLS ARE DERIVED FROM EXTRANEOCORTICAL

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The marginal zone is a heterogeneous population of cells that later forms layer one of the cerebral cortex. Recent evidence suggests that Cajal-Retzius cells and GABAergic interneurons which populate the marginal zone during development are derived from extracortical sources, but the origin of cells labelled with neocortical markers such as Tbr1 and Nfib is not known. Purpose: To investigate whether Tbr1 and Nfib-positive cells in the marginal zone originate in the neocortex or extraneocortical sources. Methods: BrdU birthdating, immunohistochemistry and in utero electroporation as well as analyses in wildtype Golli-tauGFP mice were used to address this question. Results: Staining of Golli-tauGFP reporter mice with Nfib, Reelin, Tbr1 revealed that the marginal zone and subplate can be distinguished by different sets of markers early in development (n=5 for each staining at E13 and n=3 at E16). Using in utero electroporation we specifically labelled cells from the ventricular zone of the dorsal neocortex at E11 (n=4) or E12 (n=4) and found that none of these cells contributed to the marginal zone at E16. Conclusion: Cells forming the preplate can be distinguished as destined for either the marginal zone or subplate from the earliest stages of preplate formation. Although those that form the subplate are derived from the neocortical ventricular zone, those cells that form the marginal zone are not derived from the neocortex.

POS-WED-017

CHARACTERIZATION OF INTERNEURON SUBTYPES IN THE DEVELOPING HUMAN CORTEX

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Purpose: The varying morphologies and number of interneurons in the highly evolved cortex suggest that developmental mechanisms have emerged to balance the increase in excitatory neurons. Rodent models advance our understanding of interneuron biology, however, recent evidence indicates a source of GABAergic progenitors within the cortical ventricular zone (VZ) of human and non-human primates. The definitive identity of these progenitors and maturation of locally-produced interneurons remain unclear. **Methods:** Proliferation and molecular diversity of interneurons were analysed by immunohistochemistry and in-situ hybridization in human fetal samples of 16 and 18 gestational weeks (GW). The maturation and proliferative capacity of interneurons were assessed using dissociated cultures. **Results:** Our analysis has revealed Calretinin (CR)-positive cells in the marginal zone and cortical plate (CP), punctuate staining along the radial glial fibres in the subplate and positive cells in the inner subventricular zone (ISVZ) and VZ. In contrast, Calbindin (CB)-positive cells are only found in the CP. CR-positive cells in the ISVZ/VZ have a single leading process in the tangential orientation and colocalize with the migrating neuron marker doublecortin. To assess the maturation of CR-positive interneurons, dissociated cultures of 18GW cortex were analysed up to 30 days in vitro (DIV). We found a small proportion of CR-positive cells colocalize with the proliferation marker phosphohistone H3 (pHH3) and mature over time by increasing the number and length of branches. In addition, the expression of the interneuron subtype marker parvalbumin becomes evident after 16DIV. **Conclusion:** Our results reveal the proliferative capacity of CR-positive cells and the cortical expression pattern of interneuron markers. Combined these results highlight the differing developmental profiles across species.

POS-WED-018

THE DOWN SYNDROME-RELATED GENE EURL IS IMPORTANT FOR THE PRODUCTION AND MATURATION OF NEW NEURONS WITHIN THE DEVELOPING MOUSE BRAIN

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Down Syndrome (DS) is a human genetic disorder which results from an extra copy of human chromosome 21, and this condition is detected in 1 in 700 live births. All patients diagnosed with DS suffer some form of intellectual disability, and current studies indicate that the altered expression of genes within the triplicated chromosome can negatively impact on brain development. We have identified a previously understudied gene, known as eurl, to be expressed during brain development, and our functional studies indicate that eurl controls the production and maturation of neurons during mouse brain development. Strikingly, we also find that eurl can influence the ability for neurons to form dendritic spines (membranous protrusions which constitute their contact points with other neurons, and which are the morphological correlates for memory and learning). Taken together, these studies demonstrate the validity of our approach to identify eurl as a gene that controls brain development, and provides a novel perspective on the contribution of a previously uncharacterised gene to the neurological deficits observed in patients suffering Down Syndrome.

POS-WED-019

IDENTIFICATION OF TNFAIP1/BACURD2 AS AN INTERACTING PARTNER TO RND2 WHICH CONTROLS CORTICAL NEURON MIGRATION

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Members of the Rnd family of Rho-like GTP-binding proteins, such as Rnd2 and Rnd3, have been reported to be important for controlling the initiation of neurite outgrowth and cell migration by newborn neurons of the embryonic cerebral cortex by suppressing RhoA signalling (Heng et al, 2008; Pacary et al, 2011). To address the underlying molecular mechanism for their functions, a protein-protein interaction screen was carried out to identify their putative downstream signalling pathways. These studies have led to the identification of Tnfaip1 as a binding partner to Rnd2. Co-immunoprecipitation experiments confirm their protein-protein interaction, while deletion studies map the interaction of Rnd2 to the C-terminal tail of Tnfaip1. Interestingly, Tnfaip1 recruits a RhoA degradation complex through its further association with Cul3, and our functional studies show that Tnfaip1 controls neuronal migration in vivo. Taken together, these studies highlight a putative mechanism through which Rnd2 may regulate RhoA signalling during brain development.

POS-WED-020

L-TYPE CALCIUM CHANNELS IN BASOLATERAL AMYGDALA GABAERGIC NEURONS

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Purpose: The basolateral amygdala (BLA) is a critical locus for emotional associative learning. Calcium entry through L-type voltage dependent calcium channels (L-VDCCs) has been implicated in BLA dependent-learning and synaptic plasticity. Previous studies have shown that L-VDCCs are present on glutamatergic projection neurons. Here we examined whether L-VDCCs are also present on GABAergic interneurons. **Methods:** To indentify GABAergic neurons experiments were performed on c57/b6 mice (21 - 35d) that express GFP under control of the GAD67 promoter (J Comp Neurol. 2003; 467: 60-79). Mice were anesthetized with isoflurane, decapitated, and slices were prepared. Whole-cell patch-clamp recordings and high-speed calcium fluorescence images were made from GFP positive GABAergic neurons located in the BLA. **Results:** Action potentials (APs) evoked by depolarising current injections induced a rapid rise in calcium in the soma and dendrites. The peak amplitude of the AP-evoked calcium rise was largest in the proximal dendrite and decreased with distance from the soma. The amplitude of the dendritic calcium rise showed considerable variation between neurons and was generally smaller than that observed in the more homogeneous projection neurons. A small calcium rise in the soma and proximal dendrites was also observed in response to subthreshold current injections. Both the subthreshold and AP-evoked calcium rises were enhanced by the L-VDCC activator BayK 8644. Immunofluorescence staining revealed that $Ca_V1.2$ L-VDCCs were expressed on the soma and proximal dendrites of nearly all projection neurons. However, most interneurons lacked $Ca_V1.2$ immunoreactivity. **Conclusion:** These results suggest that interneurons in the BLA express low threshold L-type calcium channels that are not encoded by $Ca_V1.2$. We propose that $Ca_V1.3$ L-VDCCs are expressed in BLA interneurons and play a role both in setting the resting cytosolic calcium levels as well as calcium influx during APs.

POS-WED-021

AT1 RECEPTORS FOR ANGIOTENSIN II NEITHER DESENSITISE NOR INTERNALISE IN SYMPATHETIC NEURONS: QUANTITATIVE REAL TIME IMAGING OF PEPTIDE BINDING TO INTACT NEURONS

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Purpose: Activated G-protein coupled receptors, including AT1 receptors for angiotensin II (AngII), generally internalise via a clathrin / β-arrestin-dependent mechanism leading to functional desensitisation. We used high speed, high sensitivity confocal microscopy to directly image AngII linked to Alexa 647 (AngII-A647) binding to AT1 receptors in transfected CHO cells and native sympathetic neurons from guinea-pig coeliac ganglion. **Methods:** We used a Leica SP5 with avalanche photodiodes and resonant scanner enabling imaging at 20 frames/s with a single pixel capture time of 0.5µs. **Results:** As expected, CHO cells rapidly internalised AngII-A647 (0.1-50nM), within a few minutes. AngII-A647 (10-100nM) bound to sympathetic neurons in distinct microdomains scattered over the neuronal surface. Fluorescence correlation spectroscopy showed minimal diffusion of bound AngII-A647 in these domains (n=20). Surprisingly, very little AngII-A647 internalised in most neurons after 60 minutes continuous exposure to the peptide. In parallel experiments, we observed AngII-A488 binding to sympathetic neurons using a high-speed cooled CCD camera whilst simultaneously recording electrical responses of single neurons with intracellular electrophysiology (n=4). AngII-A488 rapidly bound to neurons and increased neuronal excitability as long as neurons were exposed to the peptide (typically 50nM for up 20 minutes). Washout of bound peptide took up to 20 minutes, before repeated application of AngII-A647 replicated the original neuronal response. **Conclusion:** These observations show a previously unobserved behaviour of AT1 receptors revealing that they sustain a high level of on-going activity without effective desensitisation or internalisation. Such behaviour would potentiate sympathetic neurotransmission for the duration of sustained blood volume loss as a result of dehydration or haemorrhage.

POS-WED-022

EVIDENCE FOR SYNCHRONOUS MULTIVESICULAR RELEASE UNDERLYING LARGE QUANTAL POSTSYNAPTIC CURRENTS IN MOUSE AUDITORY BRAINSTEM NEURONS

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Inhibitory glycinergic synapses in auditory brainstem nuclei, including the medial nucleus of the trapezoid body (MNTB), play an important role in sound localization. Interestingly, miniature inhibitory postsynaptic currents (mIPSCs) recorded in mouse MNTB neurons exhibit an extraordinarily large range of amplitudes. **Purpose:** As the strength of synaptic inputs is dependent on the underlying quantal events, our aim was to investigate the mechanisms determining glycinergic mIPSC amplitudes in MNTB neurons. **Methods:** Whole-cell patch recordings were obtained from MNTB neurons in slices from P14-P21 mice at 33-35 degC. **Results:** Application of the calcium ionophore, ionomycin (n=7), dramatically increased mIPSC frequency without changing mean mIPSC amplitude, consistent with calcium-mediated transmitter release represented by the same population of mIPSCs. Application of gabazine (n=6) as a low-affinity glycine receptor antagonist, uniformly reduced the amplitudes of the whole population of mIPSCs, suggesting that synaptic cleft transmitter concentration is comparable for mIPSCs exhibiting very large versus small amplitudes. However, application of dextran (n=7) to increase extracellular fluid viscosity and retard transmitter diffusion out of the synaptic cleft caused a substantial increase in mIPSCs over the whole range of amplitudes. **Conclusions:** This provides direct evidence that postsynaptic glycine receptors are not saturated by quantal neurotransmitter release at glycinergic synapses in the mouse MNTB. In conjunction with our previous results, we suggest that synchronous multivesicular release, not involving compound fusion, underlies the large range in the amplitude of glycinergic mIPSCs generated in MNTB neurons.

POS-WED-023

EXCITATORY NEURONS IN LAYER III OF THE PRIMARY OLFACTORY CORTEX

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With its relatively simple trilaminar structure, the primary olfactory (or piriform) cortex is an appealing model for studying cortical sensory processing. The piriform cortex (PC) is also unusually susceptible to epilepsy. Despite much work showing the importance of neurons in layer III of the PC for seizure initiation and propagation, no studies have rigorously classified layer III excitatory neurons. **Aims:** Our aims were to quantitatively classify glutamatergic neurons in layer III of the PC and to explore their possible roles in epileptogenesis. **Methods:** Experiments used acute slices from 18-25 d-old GAD67-GFP mice in which GABAergic neurons express GFP. GFP-negative neurons were selected for whole-cell patch clamping and electrical characterisation. Electrodes contained 0.4% biocytin for recovery of morphology. **Results:** The dataset comprised 38 neurons distributed across layer III. Unsupervised cluster analysis of morphological parameters identified two major classes, pyramidal cells (n = 23) and multipolar cells (n = 15). These classes differed significantly in several electrical properties, including input resistance and kinetics of their excitatory postsynaptic currents. Both cell types received strong epileptiform excitation when GABAergic synaptic inhibition was blocked with picrotoxin. **Conclusions:** Layer III of the PC contains two morphologically distinctive classes of glutamatergic neurons that differ subtly in their electrical properties. Both appear to be equally engaged in seizure activity. Future work will examine in more detail the roles of layer III pyramidal and multipolar cells in the function and dysfunction of this olfactory circuit.

POS-WED-024

INHIBITION OF VOLTAGE-GATED SODIUM CHANNELS BY THE NOVEL µO-CONOTOXIN MfVIA

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Purpose: Nine subtypes of the pore-forming α subunit of voltage-gated sodium channels (Na_v) have been identified, each with a distinct tissue distribution, biophysical property and sensitivity to the neurotoxin, tetrodotoxin (TTX). TTX-resistant Na_v subtypes, such as $Na_v1.8$, are predominantly expressed in sensory neurons and play a role in pain transmission. In the present study, we investigate the effects of the novel µO-conotoxin MfVIA, isolated from the venom of *Conus magnificus*, on native and recombinantly expressed Na_vs. **Methods:** The effects of MfVIA were studied on Na_vs in rat dorsal root ganglion (DRG) neurons and Na_v subtypes expressed in either *Xenopus* oocytes or mammalian (HEK 293 or CHO) cells. Concentration-response curves were determined for the inhibition of depolarization-activated Na^+ currents by MfVIA and the half-maximal inhibitory concentration (IC_{50}) calculated for each Na_v subtype. **Results:** µO-conotoxin MfVIA inhibition of Na^+ currents mediated by $Na_v1.4$ gave an IC_{50} = 85 ± 21 nM, $Na_v1.5$ had an IC_{50} = 420 ± 199 nM, $Na_v1.6$ had an IC_{50} = 1.4 ± 0.1 µM and $Na_v1.7$ exhibited an IC_{50} = 2.1 ± 0.2 µM (n ≥ 5 for each Na_v subtype). $Na_v1.2$ was expressed in oocytes and MfVIA inhibition of Na^+ currents exhibited an IC_{50} = 9.7 ± 0.2 µM (n > 5). The effect of MfVIA was also examined on TTX-resistant Na_vs in rat DRG neurons and the IC_{50} was 95 ± 74 nM (n = 5). **Conclusion:** Similar to the previously characterised µO-conotoxins, MrVIA and MrVIB isolated from *Conus marmoreus*, which potently and selectively inhibit TTX-resistant Na_vs in rat DRG neurons and recombinantly expressed $Na_v1.8$ channels (Ekberg et al, 2006, *Proc Natl Acad Sci USA*. 103:17030-5), MfVIA preferentially inhibits $Na_v1.4$ and TTX-resistant Na_vs in rat DRG neurons. These data suggest that MfVIA may be potentially useful for the development of Na_v subtype-selective drugs.

POS-WED-025

LACK OF GLUTAMATE-MEDIATED RESPONSES IN IMMUNE CELLS

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Purpose: Evidence suggests the neurotransmitter glutamate may serve as a signalling molecule in peripheral tissues. In fact, several studies have demonstrated expression of glutamate receptors (GluRs) and their modulatory role in immune cells. However, the underlying mechanisms remain elusive. We investigated functional expression of GluRs in T-lymphocytes and examined their involvement in T-cell activation. **Methods:** Jurkat cells (JCs) were cultured and murine splenocytes extracted using standard techniques. Cells were activated with phorbol-ester/calcimycin or specific antigen. Membrane properties and voltage-gated currents were characterised in whole-cell configuration. Glutamate iontophoresis (10mM) was done with an AxoClamp-2B. For flow-cytometry (LSRI), the Ca^{2+}-indicator indo-1 was used. **Results:** Resting membrane potential of T-cells was variable, with activated JCs having more depolarised membrane potential than resting JCs (–34±5 *vs.* –56±2mV; $p<0.001$). Resting and activated JCs, along with activated mouse T-cells, displayed input resistance of 4.2±0.6GΩ. Membrane resistivity was higher in resting than in activated cells (5.9±0.8 *vs.* 0.4±0.1kΩcm^2; $p<10^{-9}$). None of the cells used responded to iontophoretic glutamate pulses (10ms). In JCs, a delayed rectifier K^+ conductance was identified, with activation and steady-state inactivation well-described by a Boltzmann function ($V_{1/2}$=–28.0±1.1mV and k=4.4±0.6mV for activation, –59.2±1.0mV and –4.9±0.6mV for inactivation). Activation and inactivation kinetics were voltage-dependent. These properties were unaffected by 10μM glutamate. Glutamate and NMDA (1–500μM) did not alter cytosolic Ca^{2+} in splenocytes, either at rest or upon concanavalin-A stimulation. **Conclusions:** Glutamate-mediated currents or modulation of cytosolic Ca^{2+} were not detected in T-cells and splenocytes. Although glutamate modulates voltage-gated K^+ currents in human T-cells, no such effect was observed in JCs. We hypothesise that GluRs expressed in immune cells may require priming to become functional.

POS-WED-026

ISOFLAVONES AND GABA(A) RECEPTORS

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Introduction: Ionotropic $GABA_A$ receptors are the most abundant class of inhibitory GABA receptors and are well-established therapeutic target in many CNS-related disorders. $GABA_A$ receptors display high structural heterogeneity and have large number of allosteric sites which can be modulated by a range of chemically-diverse compounds. Isoflavones are a recent addition to this growing list of modulators, and their actions on $GABA_A$ receptors are of particular interest. Methods: The modulatory activities of four isoflavones: (1) 3',7-dihydroxy-4'-methoxyisoflavone, (2) 4',7-dihydroxy-3'-methoxyisoflavone, (3) 4',5'-dihydroxy-7-methoxyisoflavone and (4) 3',4'-methylenedioxy-2-ethyl-6-propyl-7-hydroxyisoflavone) were evaluated at recombinant $\alpha2\beta2\gamma2_L$ $GABA_A$ receptors expressed in *Xenopus* oocytes using the two-electrode voltage clamp method. Results: Isoflavones 1, 2 and 3 inhibited the action of 100 μM GABA at $\alpha2\beta2\gamma2_L$ $GABA_A$ receptors with IC_{50} values of ¬105.8 [91.3, 122.5] μM, 168.8 [122.3, 232.9] μM and 787.7 [405.1, 1529] μM respectively. These three isoflavones (100 μM) also completely inhibited the enhancement of diazepam (1 μM) on the activation by GABA (100 μM). Flumazenil (1 μM), the benzodiazepine antagonist failed to reverse the inhibition caused by these isoflavones, and appeared to enhance the inhibitory action of 3 by up to 27% (p-value < 0.05). Isoflavone 4 is a potent and efficacious positive modulator (864% modulation at 30 μM) at $\alpha2\beta2\gamma2_L$ $GABA_A$ receptors. Similar to 1-3, the activity of 4 was unaffected by flumazenil (10 μM). Discussion and Conclusion: The lack of flumazenil-sensitive component in the modulatory actions of these isoflavones suggests that they do not bind to the classical high-affinity benzodiazepine binding site. In the presence of flumazenil, the activity of 3 was enhanced, implying that these isoflavones might bind to a site which is allosterically linked to the high-affinity benzodiazepine site. As there is increasing evidence pointing towards a more complex interaction underlying the different binding sites, these isoflavones can serve as tools to further study and understand the pharmacology of $GABA_A$ receptors.

POS-WED-027

USING OPTOGENETICS TO PROBE NEURONAL CIRCUITRY

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Intro: Optogenetic neuroengineering heralds a new epoch in the investigation of neuronal-circuit function. This technique utilises a range of light-activated proteins with the capacity to control neuronal activity with high spatial and temporal precision. Here we are working to establish the use of Channelrhodopsin (ChR2) to probe the neuronal circuitry of the amygdala, which is a region of interconnected nuclei known to be crucial for both the acquisition and storage of emotional memory. **Methods:** Lentiviruses driving ChR2 (hChR2-H134R) were stereotaxically injected *in vivo* into C57 mice aged p0-84 and were recovered for 2-11 weeks. Whole-cell patch clamp recordings quantified photocurrents and light-driven synaptic transmission in acute coronal brain slices. **Results:** Strong membrane-bound ChR2 expression was detectable in the target neurons of injected animals (n=11) *in vitro*, via fused-fluorophore imaging, capable of driving action potential activity following 2 weeks recovery (n=4). Light-gated synaptic transmission, driven from virally targeted terminals of the cortical (TeA) and thalamic (MGN) inputs presynaptic to the amygdala, was observed and quantified after a minimum of 3 weeks recovery (n=33). ChR2-expressing MGN projections terminated in the lateral amygdala (LA) via the internal capsule, and produced monosynaptic EPSCs (n=6). ChR2-expressing TeA projections terminated in the LA via the external capsule (n=25), and produced polysynaptic EPSCs that were often followed by strong disynaptic inhibition (n=11). **Conclusion:** These optogenetically-driven, input-specific data suggest that a significant intra-amygdaloid connectivity difference exists between TeA and MGN inputs, with downstream consequences for information processing, and previously published findings. Future experiments will continue to optogenetically investigate intra- and inter-amygdaloid circuitry, to provide critical insight into the mechanisms underlying the acquisition and storage of emotional memory.

POS-WED-028

REDUCED GIANT DEPOLARIZING POTENTIAL (GDP) EVENT FREQUENCY IN A $GABA_A$ γ2 MUTANT EPILEPSY MOUSE MODEL

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During the first days of postnatal development $GABA_A$ receptors are critical for the generation of large network oscillations termed Giant Depolarizing Potentials (GDPs). GDPs are thought to be important in the formation and maturation of the neuronal network. A syndrome-specific mouse model based on the $GABA_A$ γ2R43Q mutation recapitulates seizure types seen in humans. Seizure susceptibility in the $GABA_A$ γ2R43Q mouse is determined by a combination of developmental and acute consequences of impaired $GABA_A$ receptor function. The cellular mechanisms underlying the developmental impact are not known. **Purpose**: To investigate the developmental consequences of the $GABA_A$ γ2R43Q mutation on GDP expression in early development. **Methods**: Whole cell patch clamp recordings were made from CA3 wildtype and mutant pyramidal neurons at P3-P5. GDP network events and spontaneous postsynaptic currents were recorded in voltage clamp. **Results**: Reduced GDP event frequency was observed in the $GABA_A$ γ2R43Q epilepsy mouse model compared to wildtype littermates (n=15 and 12 respectively, P<0.05), while amplitude and width were the same in the two groups. Spontaneous synaptic events were smaller in amplitude and less frequent in the heterozygous mice (P<0.05). The $GABA_A$ receptor antagonist bicuculline blocked both GDPs and all spontaneous synaptic activity in both groups suggesting that GABAA receptors mediate a significant component of the excitatory drive in the neonatal hippocampus. **Conclusion**: The $GABA_A$ γ2R43Q mutation leads to smaller less frequent GABA mediated postsynaptic currents which may explain the reduced GDP frequency. The results also suggest that a human epilepsy mutation can influence early developmental processes in the hippocampus, and may have a long term effect on neuronal networks maturation potentially contributing to epileptogenesis.

POS-WED-029

α_1-ADRENERGIC ACTION IN PAIRS OF PYRAMIDAL NEURONES IN LAYER II/III OF RAT SOMATOSENSORY CORTEX

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When noradrenaline (NA) activates presynaptic α_1-receptors (α_1-ARs), the mEPSC rate increases due to Ca^{2+} release from stores. How NA affects evoked transmitter release requires investigation. **Purpose**: To characterise the synaptic properties of cell pairs and elucidate how NA affects evoked EPSCs. **Methods**: Whole-cell recordings from histologically verified layer II/III pyramidal neurons were obtained in 300µm thick slices of rats (P15-19) at 36±1°C. Pre- and postsynaptic neurones were current- and voltage-clamped, respectively. Presynaptic action potentials were evoked at 0.2Hz using short current pulses (~1.5nA, 3ms) with 3µM gabazine present. **Results**: The average EPSC amplitude (*n*=101) was -31.4±3.6pA with a CV of 0.6±0.1. 60% of cell pairs displayed long-term depression, which were excluded. The remainder exhibited a tight correlation between CV^{-2} and mean EPSC. From this, the average quantal current was estimated as 6.0±0.6pA with a quantal content of 4.6±0.6. This correlation suggests that EPSC size is dominated by the number of release sites but not by release probability or quantal size. Blocking store release alone reduced EPSC amplitude by 33±11%, suggesting that store Ca^{2+} increases EPSC amplitude. α_1-AR activation depressed EPSCs by 66±23% (*n*=7), which remained unchanged when stores were blocked. This finding may indicate that the depression is caused by a step early in the signalling cascade. After bursts of action potentials, NA further altered synaptic dynamics by revealing a strong facilitatory component. **Conclusion**: NA has opposite effects on EPSCs and mEPSCs suggesting differences in the molecular requirements for vesicle release.

POS-WED-030

CHARACTERISATION OF AN INTERMEDIATE DURATION INACTIVATION PROCESS OF VOLTAGE-GATED SODIUM CURRENTS IN RAT HIPPOCAMPAL CA1 NEURONS

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Purpose: To study the kinetic properties of an intermediate-duration form of sodium current (I_{Na})inactivation in rat CA1 pyramidal cells. **Methods:** Acutely dissociated rat CA1 pyramidal neurons (n=50) were voltage clamped and equilibrium and kinetic properties of several inactivation processes of different durations were observed. Intracellular papain (1mg/ml) was applied to remove "fast" inactivation. **Results:** The steady-state inactivation curve was shifted from -61.7 ±1.9 to -71.9±1.4 mV when the conditioning pulse was increased from 50 to 150 ms, suggesting a shift in the equilbrium distribution of channels to a longer latency state. Double pulse experiments revealed voltage senstive intermediate time constants of recovery from channels inactivated through the closed-open-inactivated (C-O-I) pathway of 20, 50 and 80 ms at recovery potentials of -70, -80 and -100 mV respectively (n=10). Similar duration time constants were seen in transitions from closed inactivated (CI) to closed states (C), CI->C, and in the C->CI pathway (n=10). Voltage-insenstive inactivation time constants of approximately 15 and 300 ms were seen for channels entering the inactivated state through the open state. Removal of fast inactivation with papain largely eliminated steady-state inactivation with 50 ms pre-pulse durations, but it was restored with longer pre-pulses. Macroscopic currents in papain treated cells displayed slow inactivation time constants similar to those derived from the kinetic experiments described above. **Conclusion:** Intermediate inactivation of I_{Na} is likely to play an important role in setting neuronal firing rates as well as back-propagation of action potentials, and may be a tgarget for neuromodulatory drugs.

POS-WED-031

EAAT5 IS NOT A RETINA-SPECIFIC GLUTAMATE TRANSPORTER

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It is routinely and dogmatically stated in the literature that EAAT5 is a retina-specific glutamate transporter, being expressed only by the glutamatergic retinal photoreceptors and bipolar cells and that it is absent from the brain. In the retina it serves as a presynaptic transporter with a large chloride conductance such that it acts as an inhibitory glutamate receptor. We have re-examined the dogma that EAAT5 is not expressed in the brain. We have developed and characterised a new antibody to EAAT5, which targets an intracellular loop, and have conducted PCR reactions with EAAT5-specific primers, and sequencing (5 clones) to confirm the identity of PCR amplicons. Initial results suggest that there is alternate splicing of EAAT5 in the brain that may have contributed to earlier studies not detecting such. We demonstrate by Western blotting (n=11) that EAAT5 is abundant in the brain, being present in hindbrain, midbrain and forebrain regions. Initial immunocytochemical results (3 animals) suggest a neuronal localisation as well as a glial localisation. These results lead us tentatively to suggest that EAAT5 is the missing presynaptic glutamate transporter which has been noted for at least 20 years to exist in uptake studies but which has not previously been identified at the molecular level. Whether brain EAAT5 functions to modulate glutamate release in the same way that it does in the retina awaits further investigations.

POS-WED-032

ELIMINATION OF RXFP3 SIGNALLING CAUSES REDUCED CIRCADIAN VOLUNTARY WHEEL RUNNING IN MICE

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PURPOSE: Relaxin-3 is a neuropeptide abundantly expressed in discrete populations of neurons in the brainstem, and these neurons innervate a range of forebrain areas rich in the cognate relaxin-3 receptor, the Gi/o protein-coupled receptor, RXFP3. Anatomical and pharmacological studies in rodents have implicated relaxin-3 signalling in arousal, stress, metabolism, and affective and cognitive behaviours and associated circuits. This study further investigated the role of relaxin-3/RXFP3 signalling in brain by examining the behavioural phenotype of RXFP3-deficient or knockout (KO) mice, compared to that described in relaxin-3 (RLN3)-KO mice [1]. **METHODS:** Behaviour of C57BL/6J backcrossed RXFP3-KO mice (n=24; kindly provided by Johnson & Johnson PR&D LLC, USA) was compared to wild-type (WT) littermates (n=24) in a battery of acute tests. Levels of chronic circadian voluntary running wheel activity were measured in separate cohorts of RXFP3-KO (n=15), RLN3-KO (n=15), and matching WT littermates (n=15/15). **RESULTS:** Briefly, no differences were detected between RXFP3-KO and WT littermates in the acute tests, suggesting comparable levels of motor co-ordination, spatial and recognition memory, anhedonia, fear and anxiety, sensorimotor gating, and locomotor activity in novel environments. Notably though, RLN3-KO and RXFP3-KO mice were markedly hypoactive (20-30%, $P < 0.05$) on voluntary running wheels during the dark/active phase. **CONCLUSION:** Our data confirm a similar 'circadian hypoactivity' phenotype in both strains with relaxin-3/RXFP3 signalling deficits; and highlight the potential of these mice to further elucidate the role of relaxin-3/RXFP3 in the control of arousal and other modalities under normal conditions and in relevant models of psychiatric disease. **1.** *Smith CM, Hosken IT, et al. (2011) Genes Brain Behav (in press).*

POS-WED-033

MODELLING BINOCULAR INTEGRATION IN LAYER 2/3 SIMPLE CELLS

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Purpose: Using a modelling approach we explore how the dendritic distribution of synaptic inputs onto layer 2/3 pyramidal neurons impacts on the integration of binocular information from the two eyes. **Methods:** We used the NEURON simulation environment to simulate a reconstructed layer 2/3 pyramidal neuron with passive properties. Synaptic inputs activated by the contralateral and ipsilateral eye were distributed within the basal dendrites and were independently activated in a sinusoidal manner by a non-homogeneous Poisson processes to simulate a simple cell responses to optimally oriented drifting gratings. **Results:** We tested three different distribution patterns of binocular inputs: dispersed, segregated and concentrated. For each configuration, the linearity of binocular summation was quantified by dividing the response to activation of both contralateral and ipsilateral inputs together by the linear sum of the responses to individual activation of each input. In the dispersed configuration, synapses were sparsely distributed throughout the dendritic tree with the contralateral and ipsilateral inputs positioned either randomly or close to each other. Binocular responses in these models were similar and sublinear in these cases (linearity index: 0.89). In the segregated configuration, contralateral and ipsilateral inputs were positioned on distinct dendritic branches. Notably, the spatial separation of inputs did not increase the linearity of the binocular response (linearity index: 0.89). Finally, in the concentrated model, where all the inputs were clustered on a single dendritic branch, we observed stronger sublinear summation (linearity index: 0.83). **Conclusions:** In our passive model, varying the pattern of synaptic distribution only weakly affects binocular integration, with all configurations resulting in comparable sublinear summation. We are currently investigating the impact of active nonlinear conductances.

POS-WED-034

BINOCULAR SYNAPTIC INTEGRATION IN MOUSE VISUAL CORTEX

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Purpose. To understand how binocular neurons in the primary visual cortex integrate inputs received by the two eyes. Methods. *In vivo* whole-cell current-clamp recordings were made from layer 2/3 pyramidal neurons in the binocular region of primary visual cortex of anaesthetized mice. Visual stimuli consisted of drifting sine wave gratings presented in six orientations subtending the binocular visual space. Each stimulus was presented pseudo-randomly to the contralateral eye, ipsilateral eye, and to both eyes together. Only neurons classified as simple cells ($F_1/F_0>1$) were included in the analysis. Results. Simple cells responded to drifting gratings with a sinusoidally modulated change in membrane potential at the grating temporal frequency. The average membrane depolarization during the stimulus as well as the amplitude of membrane potential modulation (peak to trough) was quantified. At the optimum orientation, stimulation of the contralateral eye evoked a larger average depolarization (6.5±0.5 mV; n=21) than stimulation of the ipsilateral eye (3.8±0.4 mV; $p<0.001$), however, the amplitude of membrane potential modulation was similar (contralateral: 5.2±0.7 mV, ipsilateral: 4.6±0.6 mV; $p=0.4$). Notably, when the stimulus was presented to both eyes together, the evoked binocular response was accurately predicted by the linear sum of the responses to activation of each eye on its own (linearity index 0.98±0.05 for average depolarization; 1.11±0.10 for membrane potential modulation). Linearity of binocular integration was also observed for non-optimally oriented gratings and was independent of stimulus contrast. Conclusion. We show that layer 2/3 simple cells compute the inputs received by the two eyes linearly. Which strategies these neurons use to achieve binocular linear summation is currently under investigation using single-cell *in vivo* two photon calcium imaging.

POS-WED-035

ORIENTATION ADAPTATION WITHOUT PLASTICITY

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Purpose: Orientation selectivity is a key characteristic of visual cortical neurons and it is first exhibited in primary visual cortex. This selectivity is not a static property of cortical neurons, but rather depends on the recent stimulus history. In particular, even a brief exposure to an oriented stimulus is known to result in shifts of orientation tuning curves. These shifts are typically interpreted as signs of short-term plasticity in primary visual cortex. This interpretation, however, ignores that visual cortical cells are part of a dynamical system with recurrent connections through which information on stimulus history becomes embedded in the neural response. We investigated whether these dynamics can explain the reported adaptation effects. **Methods:** We implemented a network model of orientation selectivity based on recurrent lateral interactions to study the temporal dynamics of orientation tuning and its dependence on stimulus history. **Results:** When presented with pairs of gratings, the model replicated the shifts in tuning curves that have been observed experimentally. No changes in model parameters or addition of new parameters were necessary to obtain these shifts; rather, they emerged naturally as a consequence of the network not reaching its steady state response during the rapid presentation of multiple stimuli. **Conclusion:** This finding has important implications for the interpretation of changes in neural responses that depend on recent history; our simulations show that such changes need not involve any plasticity and could be caused by neural population dynamics, which are slow due to the recurrent connections. These implications are not limited to the orientation tuning networks of V1 but extend to any recurrently connected neural network.

POS-WED-036

COMPUTATIONAL MECHANISMS UNDERLYING SIGNAL PROCESSING IN PRIMARY VISUAL CORTEX

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Aims. Cortical neurons are selective for orientation of stimulus, direction of stimulus motion and the spatial frequency of grating stimuli. Here we present a model of signal processing in the primary visual cortex. We ask if a simple feed-forward model can simulate the fundamental properties of the cortical cells such as direction selectivity, orientation selectivity and spatial frequency selectivity. Further, we sought to compare the population response of these properties with those found empirically. **Methods.** The feed-forward model describes a single column of cat primary visual cortex and comprises a series of processing stages from retina to the primary visual cortex. Each neuron receives convergent excitatory input from the neurons in the preceding stage, where the input is weighted with a Gaussian function centred on the recipient neuron. All neurons in the model are simulated as low-pass temporal filters to produce a generator potential. For all cells other than photoreceptors and bipolar cells, this potential is rectified to obtain action potential rate. **Results.** The model reproduces several fundamental properties of the primary visual cortex as well as the diversity of responses across population of cells. Direction selectivity originates from a small delay between on-centre and off-centre inputs to the cortex: each cortical cell produces a large generator potential in its preferred direction and a small response in the opposite direction. Orientation selectivity of the model results from the spatial separation of on- and off- inputs. Finally, population statistics in the model reproduce those found in the laboratory.

POS-WED-037

SYNCHRONY BETWEEN LGN AND VISUAL CORTICAL CELLS REVEAL THAT CORTICAL ORIENTATION SELECTIVITY CAN BE GENERATED FROM ORIENTATION BIASES

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Purpose To explore whether thalamo-cortical connectivity enables the generation of cortical orientation selectivity from the orientation biases present in responses of dorsal lateral geniculate nucleus (LGN) cells, we studied spike synchrony between LGN neurones and area 17 neurones. **Methods** In anesthetised and paralysed cats, we recorded simultaneous single unit activities from LGN and area 17 neurones having topographically overlapping receptive fields. The data was analysed for orientation tuning properties and multi-taper spectral analysis was performed to study the degree of synchrony between the two regions. **Results** Neurones in LGN showed demonstrable synchrony in the gamma band with the units in area 17 (n=10 pairs). This synchronous relationship was stronger when orientation preference of the LGN neurone was close to or similar to the orientation preference of the unit in area 17. The difference in orientation preferences and coherence metric demonstrated a statistically significant negative trend (Spearman correlation coefficient = -0.72, p = 0.01). **Conclusions** Our study shows that Gamma band synchronous activity between LGN and area 17 neurones are strongest when the orientation preferences are similar. This observation is not explainable by Hubel and Wiesel's hypothesis which predicts no such relationship between orientation preference and synchrony of LGN and area 17 neurones.

POS-WED-038

EFFECTS OF 670NM LIGHT ON MULLER CELLS IN TWO ANIMAL MODELS OF RETINAL DEGENERATION

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Purpose: Irradiation using long-wavelength, and near-infrared light is an emerging new therapy with beneficial effects in several disease models. Our previous studies have shown that pretreatment with 670nm light protects rodents from oxygen-induced (hyperoxia) and white light-induced photoreceptor damage (LD). The current study was undertaken to investigate the effects of pretreatment with 670nm light on Müller cells (MC) in these models of induced retinal degeneration. **Method:** C57BL/6J mice (n=10/group) were exposed to 9J/cm^2 of 670nm light daily for 5 consecutive days prior to being placed in 75% oxygen (hyperoxia) and retinas were collected at 0d and 14d. In the LD model, SD albino rats (n=8/group) received the same 670nm light treatment before exposure to ~1000lx continuous light for 24h and retinas were collected after 7d. Control animals had no 670nm light exposure. Tissues were processed either for histology and immunohistochemistry, or for RNA extraction and quantitative PCR for genes expressed by MC. **Results:** Immunohistochemical analysis of retinas exposed to hyperoxia and LD showed increased MC immunoreactivity to stress markers GFAP and iNOS, proinflammatory chemokine Ccl-2, and cytokine TNF-α, compared with normal retinas. Quantitative PCR analysis showed upregulation of *Lif-1* and downregulation of *Gpx-1* in stressed animals. In both models, animals treated with 670nm light showed similar, but significantly less severe ($p<0.005$), modulation of immunoreactivity and gene expression, compared with controls. **Conclusion:** The results validate the neuroprotective effects of 670nm light exposure and suggest that these effects may be mediated by boosting or supporting the functional role of MC in maintaining retinal homeostasis.

POS-WED-039

LATENCIES OF RESPONSES TO VISUAL STIMULI IN CORTICAL AREAS DM AND MT ARE RELATED TO SIZE-SUMMATION PROPERTIES

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The Middle Temporal (MT) and Dorsomedial (DM) areas are densely myelinated subdivisions of the visual cortex that receive direct projections from V1. MT, characterised by strong direction selectivity, is prominent in visual motion processing. DM neurons are narrowly tuned for orientation; however, its function is not well established. We examined the responses of 157 MT neurons and 99 DM neurons in marmoset monkeys anaesthetised with sufentanil (6μg.kg^{-1}.hr^{-1}) and N_2O (70% in O_2). Stimuli were drifting sinewave gratings of optimal orientation, presented at optimal direction of motion within rectangular windows. The lengths and widths of the gratings were varied independently. We found that, under similar stimulus conditions, the response latencies in the both areas were similar. For DM, cells that preferred larger gratings (i.e. those with larger excitatory receptive fields) tended to have longer response latencies. This relationship was evident with respect to both dimensions of the grating (length: r=0.38; width: r=0.34). Cells showing end- and side-inhibition had significantly shorter response latencies. In MT, a similar relationship existed for the dimension along the axis of motion (width: r=0.31). Thus, similar relationships were observed in areas with distinct receptive field properties, likely reflecting computations common to these early stages of cortical visual processing. Interestingly, in MT this was only observed relative to the dimension that is most functionally relevant to this area's function, possibly due to the time required for to integrate input signals from multiple V1 sub-units. This may suggest both dimensions are functionally important to DM, an area where cells are sharply tuned for orientation, suggesting a role in contour integration across space from multiple V1 inputs.

POS-WED-040

CONSEQUENCES OF ENDOTHELIN-1 INDUCED LESIONS OF THE MARMOSET MONKEY PRIMARY VISUAL CORTEX (V1): A NOVEL MODEL IN WHICH TO EXAMINE REPAIR AND REGENERATION

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Purpose: To develop and characterise a novel model of focal ischemic injury in the nonhuman primate V1. **Methods:** Endothelin-1 (ET-1) induced focal ischemia was performed on PD14 (n=2) and adult (n=1) marmoset monkeys (Callithrix jacchus). Anaesthesia was induced and maintained using inspired isoflurane (0.5-4%; adult induction: alfaxan 8mg/kg). Following craniotomy and durectomy, cortical injections of 0.33μL (1mg/ml) ET-1 (rate: 0.1μL/30s + 30s intervals) over 4 injection sites were performed, surrounding branching of the posterior cerebral artery of operculum V1. Animals were subsequently pulsed with 2x daily doses of 5-Iodo-2'-deoxyuridine (IdU; 57.5mg/kg; days 1-3) and 5-chloro-2'-deoxyuridine (CldU; 42.75mg/kg; days 7-9) post-lesion. Animals were perfused 3-weeks post-lesion by transcardial perfusion and brains were postfixed in 4%PFA before cryoprotection, cryopreservation and cryosectioning. Anatomical and cytoarchitectural changes were assessed using histology and immunohistochemistry (IHC). **Results:** Nissl substance staining, and NeuN and active-caspase3 IHC revealed the extent of the lesion, encompassing most of opercular V1, with corresponding zone of anterograde degeneration in ipsilateral LGN. V1 calcarine and adjacent V2 remained unaffected. Reactive gliosis and glial scarring were evident by marked increase in EphA4, chondroitin sulfate proteoglycan expression and GFAP-labeled reactive astrocytes at the lesion site and juxtaposing white matter. Immunofluorescent detection of thymidine analogues revealed greater numbers of IdU-positive cells at the lesion site compared to CldU suggesting higher rates of cell proliferation in the first week after injury. **Conclusion:** This new model of focal stroke in the primate is useful for examining the mechanisms of injury and repair, potentially leading to development of translational therapeutic strategies for the treatment of focal stroke in human.

POS-WED-041

CHARACTERISATION OF SECRETAGOGIN IMMUNOREACTIVE AMACRINE CELLS IN THE MARMOSET RETINA

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Purpose: Amacrine cells are the most diverse class of retinal neurones; in primates more than 25 different types of amacrine cell have been described. Here, a subset of amacrine cells in the marmoset *(Callithrix jacchus)* retina that express the Ca^{2+} binding protein secretagogin were characterised. **Method:** Retinas were either subjected to standard immunohistochemistry or pre-labelled with an antibody specific for secretagogin and subsequently immunopositive cells were injected with DiI. A total of 33 DiI labeled amacrine cells was analysed. **Results:** Secretagogin immunoreactivity was present in wide-field amacrine cells with somata in the inner nuclear layer (~60 cells/mm^2), as well as displaced amacrine cells with somata in the ganglion cell layer (~20 cells/mm^2). Labelled processes were broadly stratified in the central strata of the inner plexiform layer. Broad stratification was confirmed in individual cells that had been injected with DiI. Dendritic field diameter of DiI labelled cells was 156 ± 29 µm (mean ± SD). Secretagogin positive cells failed to co-stain with glycine or GABA (the most abundant neurotransmitters among amacrine cells), and display few morphological features of previously described amacrine cells. **Conclusion:** Taken together, our results suggest that secretagogin immunoreactive cells represent a novel type of amacrine cell in the primate retina.

POS-WED-042

INHIBITION GENERATED BY SPIKING AMACRINE CELLS SHAPED THE RECEPTIVE FIELD PROPERTIES OF MOUSE RETINAL GANGLION CELLS

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Retinal ganglion cells (RGCs) are the output cells of the eye. The signals they generate depend on the excitatory and inhibitory inputs they receive. **Purpose**: To assess the impact of excitation and inhibition on spatial-tuning properties and direction selectivity of RGCs. **Methods**: Dynamic-clamp recordings were obtained from 49 RGCs using whole-mount retinae. Light-evoked conductances recorded in response to different spots in control condition and under tetrodotoxin were injected into RGCs using a hybrid RGC-computer circuit. Digitally synthesised directionally tuned conductances, modelled on physiological responses of direction-selective RGCs were also used to assess the direction selectivity of RGCs. **Results**: Responses generated by the onset of conductances corresponding to different spot sizes displayed characteristic area-response functions of RGCs (peaked at *'small sizes'* of 150µm) which were suppressed for *'larger sizes'* conductances. In all drug-simulated conditions, the degree of suppression was significantly reduced compared to the control for spot sizes ≥ 300µm, with the strongest effect observed when direct and presynaptic inhibition were removed (~30% reduction in surround inhibition). At the offset phase, there were little or no responses, except when post-synaptic inhibition was removed, that resulted in size-tuning curves peaked at 300µm. Injection of directionally tuned conductances showed that direct inhibitory inputs modulate response strength in the preferred direction but obliterate otherwise occurring responses in the null direction. **Conclusions**: The sharpness of spatial-tuning curves of RGCs was increased by inhibition generated by spiking amacrine cells found in the inner plexiform layer. For responses at stimulus offset and stimuli moving in the null direction, direct inhibition is critical for preventing spike generation.

POS-WED-043

COLOUR VISION IN THE DAMSELFLY *ISCHNURA HETEROSTICTA*: A FUNCTION IN SEXUAL SELECTION

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Odonata (dragonflies and damselflies) possess diverse colour patterns on their bodies, and a well-known colour vision. Ischnuran damselflies, in particular, have female-limited polymorphism where andromorphs have male-like appearance, and heteromorphs have several types of colour morphs. **Purpose**: There is ample behavioural evidence that colouration plays an important role in sexual selection in context of mate choice. However, the role of colour vision and perception in sexual selection of damselflies is not known. This study investigated colour vision and intersexual recognition abilities in Ischnura heterosticta, a species with blue andromorph females and green, intermediate, and grey heteromorphy females. **Methods**: We combined electrophysiological approaches (electroretinogram and intracellular recording) with body colour reflectance measurements of the different morphs. **Results**: The intracellular recordings (n= 205 cells) showed that all morphs of I. heterosticta have four types of photoreceptors (λmax= 345, 465, 520, 540 nm), which matched the pattern generated from electroretinogram experiments. Thoraces of males (n= 25) and female andromorphs (n= 17) had similar colour reflectance with a dominant broad-band peak covering 420-510 nm and a minor peak at 330 nm, but males had a higher reflectance intensity. Heteromorph females (n=52) showed more diverse colour patterns, significantly different from those of blue morphs. Colour contrast measurements showed that males and blue andromoph females cannot be distinguished visually by this species. **Conclusion**: This study provides the first evidence that I. heterosticta has colour vision, and that the spectral sensitivities are congruent with the colour reflectance of individual female morphs. We propose that the evolution of colour discrimination in this species facilitates assessment of individual qualities via colour signals on their bodies used for sexual selection.

POS-WED-044

CONTRIBUTION OF SHORT WAVELENGTH SENSITIVE (S OR "BLUE") CONES TO VISUAL RESPONSES IN SUPERIOR COLLICULUS

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Purpose: The main output streams of the primate retina target the parvocellular (P) and magnocellular (M) layers of the lateral geniculate nucleus (LGN). Blue cones make at most only small functional contribution to P and M ganglion cells but strong contribution to many cells in the diffusely organised, evolutionary primordial, koniocellular (K) layers of the LGN. Here we asked whether blue-cones also contribute to non-geniculate output streams. **Methods:** We recorded extracellular action potentials from 38 neurons in the superior colliculus (SC) in three sufentanil-anaesthetised marmosets (Callithrix jacchus). Stimuli comprised flashed uniform fields and drifting sine gratings with defined levels of achromatic and blue-cone contrast. Functional properties were assessed from response discharges during the first 500 ms after stimulus onset. Responses were compared to our previous studies of P, M and K cells. **Results:** Robust responses to blue-cone selective stimuli were not seen in any neurons in our sample. Responses to blue-cone selective stimuli, where present, were comparable in amplitude to those seen in LGN P cells, and were not greater than predicted by residual luminance artefacts in the nominally cone-isolating stimulus. When stimulated with achromatic gratings the SC neurons showed wide variation in measured response parameters (orientation and direction selectivity, response transience, achromatic contrast sensitivity, binocularity, response rectification). The SC population is thus functionally distinct from P and M populations in LGN. **Conclusion:** We conclude that ganglion cells carrying blue-cone signals do not contribute to visually evoked activity in SC. It is possible that blue-cone signals are targeted specifically to LGN K layers rather than making widespread contribution to non-geniculate retinal targets.

POS-WED-045

KONIOCELLULAR SUPERFICIAL LAYER PROJECTING GANGLION CELLS IN MARMOSET RETINA

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Purpose: Retinal projections to the koniocellular division of the retinogenicular pathways have not been examined in detail. We compared retinal projections to the superficial layer (K1) of the lateral geniculate nucleus with those of the main koniocellular layer (K3) between the paruo- and magnocellular layers. **Methods:** Ganglion cells in two sufentanil-anaesthesised marmosets (Callithrix jacchus) were retrogradely labeled from two tracer injections in each animal. One injection was aimed at the K3 layer and a second was aimed at K1. Ganglion cell morphology was revealed by photofilling and cells classified according to their dendritic field size, morphology, stratification and branch density. **Results:** In all retinas analysed, two patches of ganglion cells were obtained. Based on the retinotopic organisation of receptive fields in marmoset lateral geniculate nucleus (White et al., 1998), the peripheral patch is thought to correspond to the injection in K1 and the central patch to the injection in the K3 layers. The peripheral patch included cells resembling the large bistratified or recursive bistratified types previously described in macaque (Dacey, 2OO4), in addition to some midget and parasol cells. In comparison, the more central patch comprised midget, parasol and small bistratified cells. **Conclusion:** Small bistratified cells project to the K3 layers whereas presumed recursive bistratified and large bistratified cells, project to the superficial layers of the koniocellular division of the lateral geniculate nucleus. This result implies a functional segregation of retinal inputs to the different koniocellular layers. Dacey DM.2004.In: Gazzaniga MS, editor. The Cognitive Neurosciences. Cambridge: MIT Press. p281-301 White AJ, et al. 1998. J Neurophysiol 80:2063-2076.

POS-WED-046

QUANTITATIVE, MORPHOLOGICAL CLASSIFICATION OF RETINAL GANGLION CELLS IN THE SOUTHERN HEMISPHERE LAMPREY, *GEOTRIA AUSTRALIS* (AGNATHA)

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Lampreys are one of two extant representatives of the earliest divergent group of vertebrates, the agnathans. The visually distinct southern hemisphere species of lamprey, *Geotria australis*, possesses the potential for pentachromatic colour discrimination as opposed to the mono- or dichromacy found in northern hemisphere species. **Purpose:** In the present study, we survey the retinal ganglion cells (RGCs) and investigate putative subtypes in the retina of the downstream migrant of *G. australis* using a quantitative, morphological approach. **Methods:** Dendritic morphology of RGCs was revealed by retrograde labelling of biocytin from the optic disc. Cells (n=74) were reconstructed digitally and subjected to morphometric analysis based upon soma area, dendritic field size, average dendritic density, average dendrite tortuousity, soma stratification and dendrite stratification. Cells were grouped by Ward's hierarchical cluster analysis and reviewed by discriminant and statistical analyses. **Results:** Nine putative RGC subtypes are hypothesised. Morphological appearance of clusters was cohesive and distinct between each cluster, while discriminant and post-hoc statistical analyses support the formation of all clusters. **Conclusion:** The diversity of putative RGC subtypes suggests visual information in lampreys may be processed in parallel streams, similar to gnathostomes. In light of the current findings, and its photoreceptive capabilities, *G. australis* may thus represent a better approximation for the upper limit of visual capabilities in this vertebrate taxa.

POS-WED-047

ELECTRICAL ACTIVATION OF INNER RETINAL NEURONS IN WILD-TYPE AND *RD1* MICE

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Purpose: Strategies to restore vision to those affected by retinal degeneration using electrical stimulation are made possible due to the survival of several classes of retinal neuron following photoreceptor degeneration. Many of these strategies have focused on activating retinal ganglion cells (RGCs) even though a large amount of signal processing occurs in the presynaptic cells of the inner retina. Utilising this existing neuronal wiring would seem the most parsimonious way to produce a coherent signal. However, because extensive remodelling of the inner retina has been shown to occur after retinal degeneration, many research efforts bypass this circuitry. We aimed to elucidate if preferentially stimulating inner retinal cells is a viable strategy for restoring vision to those with retinal degeneration. **Methods:** Responses from inner retinal cells in the mouse retinae were recorded either in retinal slices (wild-type (n>33)), or whole-mount (*rd/rd* (n>5)) configuration using whole cell patch-clamp recording during sub-retinal electrical stimulation. **Results:** In wild-type retinae, both intrinsic and synaptic potential changes were recorded from all major classes of inner retinal neurons (ON bipolar, OFF bipolar, amacrine and horizontal cells). Synaptic responses varied greatly between, and within, cell classes in terms of threshold, amplitude, polarity and latency. However, intrinsic responses were generally hyperpolarizing (n=17) and could not be blocked by 0.25mM $CdCl_2$ (n=4). Preliminary data suggests this intrinsic response may be mediated by voltage-gated K^+-channels. We also examined electrical stimulation of inner retinal cells in the retinal degenerate *rd/rd* mouse and similar electrical responses were apparent. **Conclusion:** We show that electrical stimulation directly and indirectly elicits responses in cells presynaptic to RGCs that may have an influence on the eventual signals passed to the visual cortex.

POS-WED-048

SEX-DEPENDENT CHANGES IN BDNF-TRKB SIGNALLING IN THE VENTRAL HIPPOCAMPUS OF REELIN HETEROZYGOUS MICE

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Background: Reelin plays an important role in cortical growth and lamination and its levels are significantly reduced in schizophrenia. Reelin heterozygous ($Rln^{+/-}$) mice show alterations in hippocampal synaptic function and spine density. BDNF plays a crucial role in neuronal survival and growth, and synaptic plasticity. **Methods:** Using Western blotting, we measured protein expression of BDNF and its receptor, TrkB, in the hippocampus of male and female $Rln^{+/-}$ mice (n=5-6 per group). Furthermore, the effects of gonadectomy on BDNF-TrkB signalling were analysed. **Results:** In the dorsal hippocampus, no changes in BDNF, TrkB or phosphorylation of TrkB (pTrkB) were observed in $Rln^{+/-}$ mice. In contrast, in the ventral hippocampus (VHP) levels of BDNF were increased in female, but not male, $Rln^{+/-}$ mice compared to wildtype controls. Levels of TrkB were unchanged, however pTrkB was significantly lower, again in female but not male $Rln^{+/-}$ mice compared to sex-matched controls. This decrease in pTrkB translated to further downstream effects with a significant decrease in phosphorylation of ERK1/2, again only in females. Ovariectomy had no effect in wildtype controls, but caused a significant decrease in BDNF expression in the VHP of $Rln^{+/-}$ mice. Ovariectomy reduced pTrkB in the VHP of female wildtype but not $Rln^{+/-}$ mice, where levels of pTrkB were already significantly reduced. **Conclusions:** These data show a female-specific disruption to BDNF-TrkB signalling and differential effects of ovariectomy in the VHP of $Rln^{+/-}$ mice. The results suggest an interaction between oestrogen, Reelin and BDNF-TrkB signalling.

POS-WED-049

THE RODENT ORBITOFRONTAL CORTEX: LEAKY LEARNING ERROR TERMS

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Purpose: Converging evidence suggests that the orbitofrontal cortex (OFC) is involved in flexibly updating the subjective value of stimuli. Damage to human, primate and rodent OFC functioning impairs appropriate modulation of behaviour when predictive stimuli change value e.g. persistent responding to a cue that predicts an outcome that is no longer valuable. This impaired flexible responding is usually reported with unimpaired initial learning. In contrast to this, the present study found that responding during simple Pavlovian stimulus-outcome training in rats with OFC lesions (n=7) was enhanced after extended training (21 days) but was indistinguishable from sham lesioned control rats (n=8) during early training (7 days). **Methods:** Rats were given 21 days of training (1 session/day) consisting of 16 pairings of a 15s auditory stimulus co-terminating with the delivery of a food pellet to a magazine. Magazine responding during the 15s preCS and CS periods were measured and a CS-preCS elevation score was used as an index of conditioned responding to the CS. **Results:** A repeated measures ANOVA with main factors of Group (Sham, lesion) and Day (21 days) found significant main effects of Group (p<.05), Day (p<.001) and GroupxDay interaction (p<.01). These results supported the observed pattern of data suggesting that all animals increased responding across days however the OFC lesioned group continued to increase responding after early training whereas sham animals did not. **Conclusion:** To our knowledge these are the first data to show enhanced Pavlovian acquisition after damage to the OFC. The results are interpreted in the context of the Rescorla-Wagner model by proposing a specific role for the OFC in the representation of expected outcome value.

POS-WED-050

THE PRELIMBIC CORTEX CONTRIBUTES TO THE DOWN-REGULATION OF ATTENTION TOWARDS REDUNDANT CUES

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Theories of associative learning suggest that the amount of attention a stimulus is paid determines its associability, or the degree to which it can enter into an association with an outcome (Mackintosh, 1975). Hence, an animal must attend to a stimulus in order to learn about its consequences and subsequently direct responding in its presence. Research has suggested that the prelimbic (PL) cortex, part of the medial prefrontal cortex, may be a region involved in directing behaviour away from previously relevant stimuli that no longer signal an outcome, toward stimuli that are now the current predictors of an outcome (Birrell & Brown, 2000; Killcross, Marquis, & Haddon, 2007). Consequently, the present studies aimed to clarify the role of PL cortex in attentional modulation using Pavlovian conditioning procedures. We found that pre-training excitotoxic lesions of the PL cortex led to a significantly attenuated overshadowing effect relative to sham-lesioned animals, but left intact exhibition of the blocking phenomenon ($p<0.05$). However, PL-lesioned animals subsequently exhibited significantly faster acquisition to the blocked cue and failed to exhibit a blocking of unblocking effect ($p<0.05$). We propose that observed deficits are the result of a specific disruption to the down-regulation of attention towards redundant cues. Where the ability of a stimulus to block subsequent conditioning remains intact, these animals may be utilising a mechanism that relies on the effectiveness of the reinforcer in promoting the development of an association, rather than changes in processing of the cue itself (i.e. a Rescola-Wagner (1972) US processing mechanism).

POS-WED-051

ABNORMAL RELATIONSHIP BETWEEN BDNF LEVELS AND PREFRONTAL CORTEX ACTIVITY DURING PROBABILISTIC FEEDBACK LEARNING IN SCHIZOPHRENIA

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Purpose: Brain-derived neurotrophic factor (BDNF) is an important regulator of synaptogenesis and synaptic plasticity underlying learning and memory. Reduced brain BDNF levels are found in schizophrenia, and fMRI studies examining probabilistic association learning (the gradual learning of cue-outcome associations) show reduced frontostriatal activity in schizophrenia. We hypothesised that people with schizophrenia would display an abnormal activation pattern during learning compared to controls, which would correlate with decreased BDNF levels. **Method:** Fifteen healthy controls and seven people with schizophrenia or schizoaffective disorder received a BOLD fMRI scan during administration of a probabilistic feedback learning task and a motor-control task using a 3T magnet. Analyses were performed using SPM5. Plasma was collected from whole blood in the morning prior to fMRI and blood BDNF was measured by ELISA. **Results:** There was no significant difference in plasma BDNF levels between groups. Controls performed significantly better than people with schizophrenia during probabilistic feedback learning, $t(20) = 2.82$, $p= 0.01$. Controls showed positive correlations between circulating plasma BDNF and activity in the lateral orbitofrontal cortex ($p <.01$, uncorrected), an area that is normally activated with probabilistic feedback learning. People with schizophrenia failed to show this positive correlation, and instead showed a negative correlation between plasma BDNF and activity in medial orbitofrontal cortex extending into the anterior cingulate ($p <.01$, uncorrected). **Conclusion:** People with schizophrenia showed an abnormal relationship between plasma BDNF levels and neural activity in widespread medial frontal cortex regions. These preliminary findings suggest the normal relationship between blood BDNF and task-related activity in the orbitofrontal cortex is not found in schizophrenia during probabilistic feedback learning.

POS-WED-052

ANTISACCADE PERFORMANCE IN PATIENTS WITH CLINICALLY ISOLATED SYNDROME: INHIBITORY DYSFUNCTION AND COGNITION

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Purpose: Clinically isolated syndrome (CIS) is the first episode of a demyelinating and inflammatory disease of the central nervous system, with 56% of people exhibiting a CNS related lesion developing Multiple Sclerosis (MS). Cognitive dysfunction has been consistently demonstrated in CIS patients, with the pattern of cognitive deficits being reasonably analogous to MS, and primarily involving attention deficits, reduced information processing speed, impaired working memory and executive functions. These changes are largely subclinical, and difficult to detect using current clinical assessments. Evaluation of saccadic eye movements has been found to provide a sensitive tool for assessing the subtle cognitive changes in MS. **Methods:** The present study used an antisaccade (AS) task, to assess the resolution of response conflict between volitional and automatic processes. **Results:** n=15 patients with CIS suggestive of MS, are to be compared to age-matched healthy controls. Preliminary data (n=6 CIS; n=6 control) indicate that, compared to controls, patients with CIS generate significantly more directional errors ($p<.05$) and demonstrate reduced spatial accuracy ($p=.09$). MRI and neuropsychological data was also collected, although results from this data set have yet to be analysed. It is hypothesised that error rate will be associated with damage to the ventrolateral and dorsolateral prefrontal cortices involved in stimulus appraisal, task set and decision making. Based on previous findings in MS patients, error rate will be significantly correlated with scores on the PASAT, a test of working memory, attention and processing speed. **Conclusion:** Ocular motor paradigms, coupled with MRI could provide an elegant and sensitive method of assessing cognitive change which could potentially monitor disease progression and changes due to drug intervention.

POS-WED-053

CHARACTERIZATION OF COGNITIVE FUNCTION IN TWO MOUSE MODELS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a common neurodegenerative disorder characterized by reduced cognitive function. Current therapies provide only limited relief, however stem cell therapy is a potential therapeutic option. **Purpose:** In this study two mouse models of AD were characterized behaviorally and pathologically, i.e. the triple transgenic (3xTg-AD) and an immunotoxin model, for their suitability in future stem cell experiments. **Methods:** Female 3xTg-AD and wild type controls were examined at two age groups: 13-15 months (n = 34) and 16-18 months (n = 26). For the immunotoxin model, 6-8 weeks old C57BL/6 male mice (n = 24) were treated with bilateral intracerebroventricular injections of saline or mup75-saporin toxin (0.4µg/µl/mouse) to cause cholinergic neuronal lesions. Both the groups of mice were cognitively assessed using a novel three day Morris water maze (MWM) and a traditional novel object recognition (NOR) paradigm. Afterwards, AD pathology i.e. amyloid plaques, neurofibrillary tangles or neuronal lesions were detected using immunohistochemistry. **Results:** In the immunotoxin model significant memory impairment (mean ± SEM, $p<0.0001$) in latency to reach the platform was observed in toxin treated mice compared to controls in the MWM. However, no memory loss was detected using the NOR test. Immunohistochemistry for choline acetyltransferase (ChAT) confirmed the loss of cholinergic neurons. In the 3xTg-AD model, transgenic mice showed impairment in NOR tests which correlates with the disease pathology. However, no clear learning was seen in the water maze in both younger and old age groups. **Conclusions:** Both the 3xTg-AD and immunotoxin models may be useful to detect model specific improvements in cognitive outcomes following stem cell based interventions.

POS-WED-054

AN IMPLANTABLE CHRONIC STIMULATOR FOR USE IN MICE

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Purpose Spiral ganglion neurons (SGNs) are well known to degenerate after the onset of hearing loss. There remains some debate in the literature as to whether chronic intracochlear stimulation can prevent this degeneration. Evidence has been presented in a number of different animal models including cat, guinea pig, chinchilla, and rat. Here we present the first data documenting SGNs density in ototoxically-deafened mice after chronic intracochlear electrical stimulation with our recently described fully-implantable mouse stimulator (Millard and Shepherd, 2007 J Neuro Methods. 166(2)). **Methods** Seven mice were unilaterally deafened with a neomycin-infused piece of Gelfoam® situated at the round window and chronically implanted with our stimulator. Two of these animals received chronic electrical stimulation for one month. At the end of the testing period, the animals were sacrificed and the density of SGNs quantified. **Results** Both chronically stimulated mice exhibited stable magnetically-evoked auditory brainstem responses over the duration of the chronic stimulation period with thresholds of 6.4 nC (SEM: ±0.3 nC). In these animals the chronic stimulation was well tolerated with neither animal exhibiting any sign of distress. Similar to previous results, no difference was seen in SGN densities between the cochleae of stimulated (0.0027 cells/um2 SEM: 0.00026) and implanted, unstimulated control cochleae (0.0026 cells/um2 SEM: 0.00022). **Conclusion** We present the first evidence that chronic intracochlear stimulation in neomycin-deafened mice does not promote SGN survival. We also present an implantable stimulator that provides a low-cost, convenient and efficient method to provide chronic electrical stimulation in the mouse. The combination of a fully implantable stimulator, coupled with the genetic mice models now available, make the mouse an attractive model for medical bionics research.

POS-WED-055

ACTION POTENTIAL WAVEFORM SEPARABILITY IN AWAKE UNRESTRAINED RATS

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Purpose: Extracellular multi-unit recording is a widely used technique to study spontaneous and evoked neuronal activity in awake behaving animals. In many studies these recordings are made using single-wire electrodes. In this study we aimed to test the ability of these electrodes to discriminate unit activity from multiple neurons. **Method**: Using the single-unit recording technique, coupled with iontophoresis to drive cell activity across a wide dynamic range, we studied spike waveform variability. We explored systematic differences in single unit spike waveform within and between brain regions and the influence of signal-to-noise ratio (SNR) on spike waveform similarity. We modelled spike misclassification, where a recorded event is classified as a different cell, for a range of cell densities based on neuronal recordings at different SNRs. Modelling predictions were confirmed by classifying spike waveforms with various SNRs using a leading commercial spike sorting system. **Results**: Our results show that multiple units can only be reliably distinguished under conditions of high recording SNR (> 5) and low neuronal density (< 20,000/mm^3). For SNR < 5, the probability of misclassifying spikes approaches 100% in many cases. **Conclusion**: Physiological changes and technical limitations reduce the accuracy of waveform-based spike classification. Classification errors will grow rapidly for SNRs typically used in awake behaving experiments. Studies using single electrodes should always report SNR as well as standard measures of waveform clustering reliability. Our results suggest that in studies where the SNR is low or neuronal density is high, separation of distinct units needs to be evaluated with caution.

POS-WED-056

CHARACTERIZATION OF INFRARED NEURAL STIMULATION IN *APLYSIA CALIFORNICA*

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Purpose: Infrared neural stimulation (INS) was shown to induce spatially selective neural activity without inducing a stimulation artifact or necessitating tissue contact. These characteristics make it a potentially viable alternative to electrical stimulation for applications like cochlear implants. Most INS experiments were done in mammals, but much is still unknown about the INS mechanism. Characterization of the experimentally-tractable nervous system of the mollusk, *Aplysia californica*, will provide insight into the underlying processes of INS in order to optimize laser design and parameters. **Methods:** Unmyelinated nerves of *Aplysia* were used to test stimulation thresholds in response to varying pulse durations (350µs-20ms), repetition rates (0.5-10 Hz), wavelengths (1.865-1.875 µm), and ambient temperatures (0-38°C) (N=3-6 for each parameter level). Nerve recordings were taken while stimulating buccal nerve 3 using pulsed infrared light. At each parameter value, stimulation thresholds were calculated as the effective dose (ED50) generated by a probit regression. **Results:** No change in stimulation thresholds were observed at repetition rates ranging between 0.5-10 Hz. Stimulation at pulse durations ranging from 3-10 ms showed no change in threshold, but threshold decreased below and increased above that range. Studies showed that a wavelength of 1.875µm was more efficient for inducing action potentials than at 1.865µm. Investigations of the ambient temperature challenged previous work by showing that threshold increased at lower temperatures (0C). Complex behavioral patterns were induced using INS in neural networks providing new directions for future clinical devices. **Conclusion:** Having characterized INS and induced behavioral patterns in *Aplysia*, we believe this is a useful model for further studies on the physiological mechanism and optimal laser parameters of INS.

POS-WED-057

CONNECTIONS OF PREMOTOR AREAS IN THE MARMOSET MONKEY

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We investigated the afferent connections of three subdivisions of premotor area 6 (6DC, 6DR and 6Va) using retrograde fluorescent tracers in marmosets (n=12) anaesthetised with Alfaxan (10 mg/kg). In addition, some injections included area 8C, a recently recognised strip of cortex inserted between areas 6DC and 6Va. In common with motor areas, 8C lacks a distinct granular layer. All subdivisions explored receive anatomical input from cingulate areas 23 and 24, multiple posterior parietal areas (PE, PF/PFG, PG, and the intraparietal areas), and caudal temporal extrastriate areas. In addition, dorsocaudal area 6 (6DC) receives strong input from the primary motor area (area 4), and modest inputs from somatosensory areas S2, 1, 2, 3a and 3b, and posterior cingulate area 31. Dorsorostral area 6 (6DR) has connections with prefrontal areas and with retrosplenial/ posterior cingulate areas (29, 30 and 31), but receives virtually no input from area 4 or somatosensory cortices. Ventral premotor cortex (6Va) has input from area 4, somatosensory and prefrontal areas (including gustatory area PrCO), but no input from areas 29, 30 and 31. Injections invading area 8C produced results roughly comparable to those in area 6DC, but revealed stronger inputs from PF/PFG, and weaker inputs from PE/ PEc. In addition, area 8C receives connections from a motion-sensitive complex of superior temporal areas (including MT and MST), as does area 6DR. The results show that area 6DR has cognitive, limbic and visual connections, whereas 6DC and 6Va are more directly involved in sensorimotor control. The connections of area 8C suggest that it may be a distinct premotor region concerned with eye-head movement while tracking a moving object.

POS-WED-058

CEREBELLAR ZONAL ORGANIZATION AND FUNCTION

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Purpose: The main information processing part of the cerebellum is its cortex, and its sole output is via the Purkinje cells which directly inhibit neurones of the cerebellar nuclei. The cortico-nuclear pathway is thus central to cerebellar operation. An important organizational principle of the cerebellar cortex is its division into a series of rostrocaudally-oriented zones. A fundamental gap in our knowledge of cerebellar information processing is the effect of Purkinje cell activity on nuclear cell output, particularly in relation to the zonal organization. The aim of the present study was to investigate the subzonal topographical organization of the C1 zone in the rat in relation to its anatomical connectivity and physiological responses. **Methods:** In 28 anaesthetized adult Wistar rats percutaneous electrical stimulation of different parts of the ipsilateral hindpaw was used to evoke local field potentials in the C1 zone in copular pyramidis and nucleus interpositus anterior. In anatomical studies, the evoked responses were used to guide nanoinjections of bidirectional tracer (BDA and beads) in the cerebellar cortex to label cells in the inferior olive and Purkinje cell terminals in nucleus interpositus anterior. **Results:** Anatomical results suggest that a detailed subzonal topography is present within the C1 zone in which physiological responses and olivo-cortico-nuclear connections are correlated. In physiological studies, simultaneous cerebellar cortical and nucleus interpositus anterior field potential recordings revealed a positive correlation in the covariation of the amplitude of the responses to hindlimb stimulation (r^2=0.5526, P<0.0001), with paired single unit recordings between Purkinje cells and its target nuclear cell showing a complex pattern of interaction that is dependent on climbing fibre activity. **Conclusion:** In sum, a detailed subzonal topography is present within the C1 zone; the physiology suggests that a point-to-point anatomical topography is functionally significant.

POS-WED-059

THE TRANSCRIPTION FACTOR MRF IS REQUIRED FOR MYELIN MAINTENANCE IN THE ADULT CNS

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Purpose: Myelin gene regulatory factor (MRF) is an essential transcriptional regulator required for myelination in the developing CNS. Mice lacking MRF within the oligodendrocyte (OL) lineage die at 3 weeks postnatal due to failure of OL maturation and myelination. Here we investigate whether MRF also has an ongoing role in the maintenance of myelin in the adult CNS. **Methods:** We generated an inducible conditional knockout (iCKO) mouse strain (MRFfl/fl; PLP-CreERT +ve) in which MRF can be ablated in myelinating cells via tamoxifen (4OHT) administration. Adult (8 weeks) MRF iCKO and control (CreERT –ve) mice were given intraperitoneal injections of 1mg 4OHT per day for 5 days. Overt signs of demyelination were assessed using a modified EAE disease severity scale and the rotarod test of motor coordination. Demyelination and tissue responses were investigated by electron microscopy and immunohistochemistry for key markers in the spinal cord, optic nerve and corpus callosum. **Results:** iCKO mice showed decreased rotarod performance and clinical symptom onset from 5 weeks post-tamoxifen, progressively worsening to a peak clinical deficit at 8 weeks. Clinical symptoms included ataxia, shivering and progressive paralysis. Electron microscopy revealed a 57% reduction of myelinated axons in the spinal cord of iCKO mice when compared to control animals (p<0.01), as well as myelin debris and significant vacuolization. iCKO mice displayed significant increases in the density of damaged (APP+ve) axons (p<0.001) and CD68+ve activated microglia (p<0.001) in all examined tissues. **Conclusion:** Our findings demonstrate that MRF is a critical transcriptional regulator essential for both developmental myelination and myelin maintenance in the adult CNS. Future experiments will elucidate the role of MRF in disease and therapeutics.

POS-WED-060

INTRACELLULAR SIGNALLING PATHWAYS THAT REGULATE OLIGODENDROCYTE MYELINATION *IN VITRO*

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Purpose: Normal myelin development is crucial for proper central nervous system (CNS) function. However, the precise nature of the signals regulating CNS myelination remains unclear. We have identified a key role for brain-derived neurotrophic factor (BDNF) in promoting myelination, through oligodendrocyte-expressed TrkB receptors. Here we investigate the downstream signalling pathways regulated by BDNF to promote myelination. **Methods:** *In vitro* myelination assays of dorsal root ganglion neurons and oligodendrocyte precursor cells (OPCs) were treated with BDNF and either immunostained for MBP, or lysed and analysed by Western blot. **Results:** To investigate the intracellular signalling pathways regulated by BDNF to promote myelination, we screened candidate signalling pathways. We found activity of the MAPK/Erk signalling pathway positively correlated with BDNF-induced myelination *in vitro* (n=3). OPCs infected with mutant constructs regulating MAPK signalling indicate MAPK activation is critical for myelination *in vitro* (n=3). Furthermore, over-expression of Erk1 or Erk2 in OPCs suggests Erk2 is the MAPK playing a key role to enhance myelination (n=3). Erk2 is a proline-directed serine/threonine kinase and is known to exert some of its effects through direct transcription factor phosphorylation. A serine or threonine followed by a proline (S/T-P) is the minimal consensus sequence for phosphorylation by Erk2. We carried out an *in silico* analysis which identified this consensus sequence in several oligodendrocyte-specific transcription factors required for myelination. We are currently undertaking immunoprecipitation experiments to investigate whether an interaction between Erk2 and these transcription factors occurs *in vitro*. **Conclusion:** Collectively, this work identifies a novel role for Erk2 signalling within oligodendrocytes that regulates CNS myelination, possibly via activation of specific transcription factors.

POS-WED-061

EVALUATION OF INTEGRIN CD11B AS TARGET FOR RECEPTOR-MEDIATED NON-VIRAL GENE TRANSFER INTO MICROGLIA

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Purpose: Microglial cells are the resident immune cells of the central nervous system and are activated upon pathological insult. Microglial activation has been linked to neuroprotection and neurotoxicity in various neuropathologies. Targeted gene delivery via antibodies may be useful to manipulate microglial function on a genetic level in order to understand the role of microglia. Here we investigate the microglial CD11b integrin receptor as target for gene delivery using a non-viral antibody bioconjugate ('immunogene'). **Methods:** Primary microglial cells (>98% pure) were collected by shaking mixed glia cultures obtained from neonatal rat brains. Monoclonal antibodies were tagged with fluorescent dyes to study internalization and receptor-mediated uptake via confocal imaging. Specificity of CD11b-targeting OX42 antibody for microglia was determined in double-labelling experiments *in vitro* and *in vivo*. An immunogene was synthesized and a gel retardation assay performed. **Results:** OX42 antibody targeting CD11b was rapidly internalized in microglia (n≥72 cells). Uptake of OX42 was receptor-mediated, because a control antibody (X63) was not internalized (n≥15 cells). Double-labelling with anti-Iba1 and anti-GFAP antibodies confirmed the specificity of CD11b for microglia in mixed glia culture (n≥20 cells) and *in vivo* after injection of OX42 and X63 into rat brain (n=2). An OX42-immunogene was constructed and shown to bind plasmid DNA. **Conclusion:** The microglial CD11b integrin receptor may be a useful target to transfect microglial cells based on its ability to internalize OX42 antibody and its specificity for microglia *in vitro* and *in vivo*. The OX42-immunogene may be valuable for non-viral gene transfer and study of microglia in neuropathologies.

POS-WED-062

RESVERATROL AND APIGENIN ALTER THE INFLAMMATORY RESPONSE OF LIPOPOLYSACCHARIDE AND IFN-γ-ACTIVATED MICROGLIA AND MACROPHAGES

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Purpose: Microglia are immune effector cells contributing to neurotoxicity in neurodegenerative diseases. Recent studies support the hypothesis that infiltration of peripheral macrophages into the brain might also contribute to neuroinflammation in Alzheimer's disease. Following activation, microglia and macrophages differ in their production of cytokines, cell surface immune antigen expression, and ability to induce immune responses. Understanding the differences in the release of pro-inflammatory factors by microglia and macrophage cells is important for understanding the mechanism of potential drugs to treat inflammatory and neurodegenerative disorders. **Methods:** RAW 264.7 macrophages, C8B4 microglia and primary murine microglia cells were activated with lipopolysaccharide (LPS) combined with interferon-γ (IFN-γ) and levels of 23 cytokines were measured using a cytokine array. Cells were treated with resveratrol and apigenin, two anti-inflammatory compounds that have been suggested as potential anti-neurodegenerative. Nitrite levels, as marker of inflammation, were measured by the Greiss method. **Results:** Exposure of cultured RAW 264.7, C8B4 and primary microglia to LPS and IFN-γ increased nitrite levels from below 3 μM up to 40 μM in RAW and 10 μM in microglia cells (n=3). Interestingly, RAW activation resulted in greater interleukin release, whereas C8B4 cells showed greater chemokine release. (n=3) Drug treatment with either 25 μg/ml resveratrol or 35 μg/ml apigenin lead to a decrease in nitrite of 50% in RAW cells (n=3). **Conclusion:** These findings show that microglia and macrophages respond to activation by inflammation specific stimuli by releasing a different cytokine profile. A potent suppressive effect of resveratrol and apigenin on pro-inflammatory responses of microglia and macrophages was identified, suggesting a therapeutic potential for these compounds in neurodegenerative diseases accompanied by microglial activation and macrophage infiltration.

POS-WED-063

EXPRESSION OF GFAP SPLICE VARIANTS IN THE RODENT CENTRAL NERVOUS SYSTEM

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Purpose: Astrocytes can be identified in the brain by the expression of glial fibrillary acidic protein (GFAP). GFAP is expressed in the core or cytoskeleton of the astrocyte and its function is to provide stability to the cell processes. The expression of GFAP is altered in many diseases that affect the central nervous system (CNS), including multiple sclerosis, stroke, Alzheimer's disease and traumatic brain injury. Novel isoforms of GFAP (GFAPε and GFAPκ) have recently been identified and may play an important role in development or disease. GFAPε and GFAPκ differ from GFAPα in the C' terminal region of the protein and have been associated with destabilising the formation of GFAP polymers. **Methods:** We have generated polyclonal antibodies against GFAPα, GFAPε and GFAPκ and examined the expression of GFAP splice variants using western blotting and immunohistochemistry in CNS tissues from rats, mice and guinea pigs (N=9). **Results:** Immunohistochemistry revealed that GFAP splice variants co-localized with regular GFAP, detected using a commercial polyclonal antibody from DakoCytomation. GFAPα, GFAPε and GFAPκ were detectable by western blotting in the majority of tissues examined (cortex, hippocampus, thalamus, brain stem, cerebellum, spinal cord and retina). Western blotting revealed slightly different expression profiles of the splice variants, with GFAPκ weakly expressed in the retinas of rats, mice and guinea pigs and GFAPε strongly expressed in guinea pig retina. **Conclusion:** This study is the first to demonstrate the protein expression of GFAP splice variants. Future studies will examine what role these splice variants might play in development and disease.

POS-WED-064

BDNF PROMOTES CNS MYELINATION IN VIVO VIA OLIGODENDROGLIAL TRKB RECEPTORS

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Understanding the mechanisms of myelin development could provide key clues to identify promyelinating factors that could potentiate remyelination in demyelinating diseases, such as Multiple Sclerosis. During development, CNS myelination is achieved by the oligodendrocyte which extends a multi-lamellar membrane sheath around axons. The precise mechanisms required for this myelination process are yet to be fully determined. Previously, the Brain Derived Neurotrophic Factor (BDNF) knockout was shown to suffer CNS hypomyelination, suggesting it exerts a regulatory role in CNS myelination. Using *in vitro* myelination assays, co-culturing dorsal root ganglion (DRG) neurons with oligodendrocyte precursor cells (OPCs), we identified that BDNF directly promotes oligodendrocyte myelination (n=3). Further pharmacological experiments suggest that the promyelinating effect of BDNF is via activation of oligodendrocyte-expressed TrkB receptors (n=3). To verify these findings *in vivo*, we have generated a conditional knockout of TrkB in oligodendrocytes ($TrkB^{fl/fl}$ MBPcre). Developmental analyses indicate that $TrkB^{fl/fl}$ MBPcre mice exhibited a significant reduction of myelin protein expression and myelination of the axonal tracts in the CNS (n=3). The reduced myelination in $TrkB^{fl/fl}$ MBPcre mice is not due to a reduction of oligodendrocyte number (n=3), but a reflection of thinner myelin, as determined by ultrastructural analyses. The $TrkB^{fl/fl}$ MBPcre mice also display a significant increase in OPC number (n=3), which could be an endogenous response of the reduced myelination. In summary, the deletion of oligodendroglial TrkB results in reduced myelination and a proliferative response amongst oligodendrocyte progenitors, indicating that BDNF has a pro-myelinating role in the CNS. In the future, we aim to investigate the mechanism for the increased OPC proliferation in $TrkB^{fl/fl}$ MBPcre and whether BDNF-TrkB has a potential in remyelination.

POS-WED-065

COCHLEAR LESIONS REDUCE THE PROLIFERATION OF GLIAL CELL PRECURSORS DURING A SENSITIVE PERIOD IN THE RAT AUDITORY BRAINSTEM

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Purpose: Deafferentation experiments can result in neuronal loss, re-organization of synaptic connections and alterations in the size of brain structures. Here, we tested the hypothesis that cochlear lesions lead to a decrease in the proliferation of glial cell precursors in the medial nucleus of the trapezoid body (MNTB), a brainstem nucleus involved in sound localization. **Methods:** To examine cell proliferation in the MNTB, we acutely injected the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU) in rat pups at postnatal day (P) 3, P9 and P21, and examined brainstem sections using multi-fluorescence labeling (n=6 rats). We found that about 40-70% of EdU+ cells were NG2+, a molecular hallmark of glial cell progenitors. In addition, the density of proliferating cells changed with age (EdU+ cells/mm^2: 200 ± 8 at P3; 239 ± 14 at P9; 26 ± 4 at P21), suggesting that cell proliferation is developmentally regulated. Hence, we examined the time course of cell proliferation between the ages of embryonic day 19 and P31 (n=43). We found that the density of EdU+ cells increased from perinatal ages, reached a plateau from about P2-P12, declined sharply between P13-P15 and remained at low levels until P31. The developmental decrease in cell proliferation was strongly correlated with the maturation of hearing, suggesting a link between the auditory periphery and the growth of the MNTB. Hence, we performed bilateral cochlear lesions at P1, P5, P9, P12 and P15, and examined cell proliferation one day after the lesion. We found that cochlear lesions led to a decrease in cell proliferation in experimental animals *versus* sham controls (n=10). **Conclusion**: These results provide evidence for a sensitive period in postnatal development when deafferentation affects the proliferation of glial cell precursors, possibly leading to alterations in the size of the auditory brainstem.

POS-WED-066

MORE THAN JUST REUPTAKE INHIBITORS: A COMPARATIVE EXAMINATION OF THE ANTI-INFLAMMATORY EFFECTS OF SSRI AND SNRI ANTIDEPRESSANTS ON MICROGLIA

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Selective serotonin and serotonin norepinephrine reuptake inhibitors (SSRI; SNRI) are the frontline pharmacological treatment options for major depression. While these drugs have long been assumed to exert their antidepressant effects because of their ability to alter central monoamine levels, they have also been shown to exert anti-inflammatory effects. The evidence for this originally only related to cells of the peripheral immune system, but recently it has become apparent that these drugs can also exert anti-inflammatory effects on microglia, the principal cells within the CNS that regulate and respond to inflammatory factors. From a pharmacological standpoint, much critical information remains unknown. In particular, the relative efficacy of these drugs in modulating microglial responses is yet to be determined. To address these issues, the current study evaluated the ability of five different SSRIs (fluoxetine, sertraline, paroxetine, fluvoxamine and citalopram) and one SNRI (venlafaxine) to suppress microglial responses to an inflammatory stimulus. Specifically, we examined their ability to alter tumor necrosis factor-α (TNF-α) and nitric oxide (NO) production after 4 and 24 h stimulation with lipopolysaccharide. Our results indicated that the SSRIs potently inhibited microglial TNF-α and NO production. We then investigated whether these effects might involve either β-adrenoceptor or cAMP signalling. Using the protein kinase A inhibitor Rp-CAMPs, we found evidence to suggest that cAMP signalling may be partially involved in regulating the anti-inflammatory response. Findings such as these could suggest that antidepressants may owe at least some of their therapeutic effectiveness to their anti-inflammatory properties, results which may in the future prove useful to improving both treatment options and outcomes for those placed on antidepressant treatment.

POS-WED-067

HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP-1) REGULATES EXOCYTOSIS VIA MULTIPLE MECHANISMS

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Huntingtin-associated protein 1 (HAP-1) has a greater binding affinity for mutant huntingtin, a key player in Huntington's disease, than normal huntingtin. Subcellular localisation and protein interaction data indicate that the HAP-1 may be important in vesicle trafficking and cell signalling. However, no physiological evidence exists to verify this possibility. We measured exocytosis using carbon-fibre amperometry on chromaffin cells cultured from HAP-1$^{-/-}$ (KO), and HAP-1$^{+/+}$ (WT) mice. Levels of exocytosis in WT (102.2 ± 10.2 exocytotic events, n=29) cells are significantly greater than in KO cells (60.4 ± 7.1, n=35; $p<0.01$). The duration of transient fusion pore opening is prolonged in KO cells (3.0 ± 0.1 ms) compared to WT (2.3 ± 0.1 ms, $p<0.05$) cells indicating that HAP-1 may stabilize the formation of the fusion pore. The size of the RRP is smaller in KO cells (19 ± 5.3, n=7) compared to WT cells (54.4 ± 8.9, n=7, $p<0.01$). Also, the amount of glutamate released from KO cortical brain slices was significantly smaller (0.03 ± 0.005uM/mg protein, n=4 animals) compared to WT slices (0.06 ± 0.006, n=4 animals, $p<0.01$). Real-time PCR also indicates the downregulation of multiple exocytosis-related genes in KO cells and immunocytochemical analysis indicates mislocalization of a transcriptional repressor in KO cortical neurons. Using a proteomics approach, novel interactions between HAP-1 and known trafficking-related proteins have also been discovered. Our study reports a novel role of HAP-1 as a regulator of neurotransmitter release by influencing the rate of exocytosis, the dynamics of fusion pore opening, the size of the readily releasable pool (RRP) of vesicles released immediately upon stimulation, transcription of exocytosis genes and via binding to proteins involved in trafficking.

POS-WED-068

PROLACTIN SIGNALS VIA THE STAT5B BUT NOT THE STAT5A PATHWAY IN THE MOUSE HYPOTHALAMUS

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Purpose: Prolactin acts at multiple targets throughout the body including the mammary gland, heart, liver, muscle and brain. Upon binding to its receptors, prolactin signals through the phosphorylation and thus activation of signal transducer and activator of transcription 5 (Stat5). There are two very similar Stat5 isoforms termed Stat5a and Stat5b, which are selectively activated by prolactin in specific tissues. Various brain regions including the hypothalamus are prolactin responsive but the Stat5 isoform involved in these actions is unknown. Methods: Immunohistochemical and Western blot analysis were used to determine the expression and activation of Stat5a and Stat5b throughout the hypothalamus in adult wild-type and Stat5b-deficient mice. Both groups (n=4) were pre-treated with 5mg/kg of bromocriptine to suppress endogenous prolactin levels followed by administration of 10mg/kg ovine prolactin for 45min. Results: Stat5a and Stat5b were expressed throughout the hypothalamus of wild-type mice. As expected only Stat5a was detected in Stat5b-deficient mice, although there was a marked reduction in its expression compared to wild-type mice. When stimulated with prolactin, phosphorylated Stat5 was observed in the hypothalamus of wild-type but not Stat5b-deficient mice. Thus while Stat5a was still expressed in the Stat5b-deficient mice it was not phosphorylated in the hypothalamus in response to prolactin. Conclusion: These observations indicate that Stat5b but not Stat5a exclusively mediates prolactin's hypothalamic actions. Despite the similarity between the two Stat5 isoforms, Stat5a was unable to compensate for the absence of Stat5b suggesting that each isoform exhibits a unique biological activity.

POS-WED-069

TDP-43 PATHOLOGY APPEARS TO BE A COMMON END STAGE PHENOMENON IN MOTOR NEURON DISEASE (MND)

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Background: Motor neuron disease (also known as amyotrophic lateral sclerosis) is an adult-onset neurodegenerative disorder characterized by the loss of both upper and lower motor neurons. TDP-43, a DNA/RNA binding protein, is a major component of pathological inclusions in MND cases. Presence of mutations in the gene encoding TDP-43 supports a causative link between TDP-43 dysfunction and neurodegeneration. However, the mechanisms through which TDP-43 causes MND are poorly understood. **Objectives:** Establish neuronal (NSC-34) and non-neuronal (lymphocyte) cell models of MND and to investigate the possible effect of TDP-43 mutations on cell division and cytotoxicity. Also, to determine if TDP-43 pathology is present in familial MND cases that are negative for mutations in known MND genes. **Methods:** Both neuronal and non-neuronal cell lines were investigated using immunohistochemisty, immunofluoresence, western blot, trypan blue and MTS assays. Aggregates in MND patient tissues were investigated using immunohistochemistry and immunofluorescence. **Results:** TDP-43 aggregates were observed in familial MND cases that were negative for mutations in known disease causing genes (SOD-1, FUS, and TDP-43). In both neuronal and stressed non-neuronal cells, TDP-43 was found to be pathologically modified. TDP-43 mutations caused toxicity in neuronal cells but the apoptotic pathophysiology in non-neuronal cells was not observed under basal or stress stimulated conditions. **Discussion and Conclusion:** TDP-43 pathology is a common feature of familial MND and the same pathology (i.e. redistribution, aggregate formation and ubiquitination) is observed in transfected neuronal and stressed non-neuronal cells.

POS-WED-070

SECRETORY VESICLE EXOCYTOSIS IS ABROGATED IN MUCOPLYSACCHARIDOSIS IIIA (MPS IIIA)

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Purpose: Mucopolysaccharidosis IIIA (MPS IIIA) is a lysosomal storage disorder caused by a deficiency in the activity of sulphamidase resulting in the accumulation of partially degraded heparan sulphate, gangliosides GM2 & GM3 and cholesterol. In this study we have used chromaffin cells to examine neuronal function in MPS IIIA. **Methods:** Chromaffin cells were cultured from age matched control and symptomatic (>15 weeks of age) MPS IIIA mice. The release of catecholamines was measured using carbon fibre amperometry. For calcium M) and then stimulatedμimaging, chromaffin cells were loaded with Fluo-4AM (5 for 60 seconds in Krebs buffer containing 70mM K+. Electron microscopy was used to define cellular ultrastructure. **Results:** There was a statistical decrease in the number of exocytic events in MPS IIIA (60 ± 16 events; n=3 mice), when compared to controls (105 ± 22 events, $p<0.05$, n=3 mice). There was no difference in the spike area or the kinetics of individual amperometric spikes in MPS IIIA cells when compared to controls. Similarly there was no significant change in foot signal area, amplitude or duration, or calcium entry and handling. Control chromaffin granules were characteristically round whereas they were both round and elongated in MPS IIIA. **Conclusion:** We show for the first time that secretory vesicle exocytosis is abrogated in MPS IIIA. This may have implications for the pathogenesis of neurological degeneration in MPS IIIA, as a decrease in neurotransmitter release could explain the observed memory and learning problems.

POS-WED-071

ROLE OF THE PRESYNAPTIC PLASMA MEMBRANE IN SORTING LOCAL FROM RETROGRADE VESICULAR TRAFFICKING PATHWAYS

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Purpose: Neurons have the ability to internalise and process endocytic cargoes at the level of the presynaptic nerve terminal. The fate of these cargoes is either to remain local (synaptic vesicles) or to be transported towards the cell body via retrograde transport. We aimed to investigate how local and retrograde trafficking pathways are separated by examining whether the sorting takes place at the level of the plasma membrane (internalisation in distinct compartments) or in a sorting endocytic station. To visualise local and retrograde cargoes we used botulinum neurotoxin type-A heavy chain (BoNT/A-Hc) and cholera toxin B-subunit (CT-B) respectively. BoNT/A is known to enter synaptic vesicles (local pathway) and cholera toxin has been used as a neuroanatomy retrograde tracer and shown to be retrogradely transported. **Method:** Cultured hippocampal neurons were incubated with appropriately labeled BoNT/A-Hc and CT-B and processed for either confocal or electron microscopy. Fluorescence imaging was also carried out at the neuromuscular junction. **Results:** Fluorescence microscopy revealed a significant level of colocalisation between BoNT/A-Hc and CT-B in hippocampal presynaptic nerve terminals. However, electron microscopy showed that this colocalisation was only apparent, as the majority of the markers were located in distinct vesicles (only 5.3±1.2% of BoNT/A-Hc vesicles also contained CT-B). These results were supported by imaging at the neuromuscular junction, which showed CT-B is taken up in regions spatially separated from active zones, where BoNT/A-Hc is internalised. **Conclusion:** Our results suggest that the presynaptic plasma membrane significantly contributes to the separation between local and retrograde endocytic pathways. A small number of cargoes containing both toxins were detected suggesting that endocytic sorting stations do also play a role in separating local from retrograde material.

POS-WED-072

THE ROLE OF NDFIP1 IN REGULATING PTEN UBIQUITINATION AND TRAFFICKING IN SITU

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Ubiquitination is a post-translational modification used to target proteins for either trafficking or degradation via 26S proteasomes or lysosomes. E3 ligases conjugate ubiquitin peptides following target recognition and binding. This targeting can be enhanced by adaptor proteins such as Ndfip1 that recruit Nedd4 E3 ligases. Recently we showed that Ndfip1 plays an important role in the ubiquitination of PTEN, a well-known tumor suppressor protein with multiple roles including synapse development. **Purpose:** To investigate the role of Ndfip1 in regulating PTEN ubiquitination and trafficking in situ. **Methods:** Interactions between Ndfip1 and PTEN as well as PTEN ubiquitination were investigated using Bimolecular Fluorescence Complementation (BiFC). This method is based on the complementation between fragments of enhanced Yellow Fluorescence Protein that have been attached to target proteins. PTEN and Ndfip1 BiFC constructs and various subcellular markers were transfected into COS and MEF cell lines and protein interactions as well as ubiquitination were imaged using confocal microscopy. **Results:** 1) The cytoplasmic region of Ndfip1 interacts with PTEN within specific organelles of the cell. 2) Ubiquitinated PTEN has a different distribution pattern compared to PTEN in general. 3) Ubiquitinated PTEN was found to be distributed around the nucleus, and co-localizes with cis-Golgi Network, ER, early endosomes, recycling endosomes, lysosome, but not late endosomes. 4) A fraction of the ubiquitinated PTEN was also found to be translocated to the nucleus of the cell. **Conclusion:** Using BiFC, we observed an interaction between Ndfip1 and PTEN that is associated with PTEN ubiquitination. Ubiquitinated PTEN was found to change subcellular location and distribution within nuclear and peri-nuclear regions. These findings suggest that PTEN ubiquitination is a major regulator of PTEN subcellular localization, with major implications for PTEN function during development and disease.

POS-WED-073

ACTIVITY-DRIVEN EXPANSION OF NEUROSECRETORY CELL FOOTPRINTS IS MEDIATED BY ACTIN REMODELING NOT EXOCYTOTIC EVENTS

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Neurotransmitter and hormonal release relies on the timely exocytotic fusion of secretory vesicles with the presynaptic plasma membrane. Such membrane addition was hypothesized to lead to the enlargement of the nerve terminal footprint. However, secretagogue stimulation promotes actin remodeling that may also contribute to this effect. **Purpose:** To assess the precise contribution of activity-dependent actin remodeling and exocytic fusion events to changes in footprint surface area. **Methods:** We used fusion-incompetent PC12 cells stably knocked down for Munc-18-1 and -2 (DKD) to determine the respective roles of exocytic fusion events and actin remodeling imaged by TIRF microscopy during secretagogue stimulation. Re-expression of Munc18-1-WT in DKD PC12 cells was used to restore fusion competency. **Results:** Inspite of the virtual absence of exocytic events in DKD PC12 cells, the footprint size increase (1.86+/-0.56 fusions events in response to barium stimulation (n=11)) was comparable to that found in DKD cells re-expressing Munc18-1 (8.1+/-1.1 fusion events (n=14)), demonstrating that it is independent of exocytic fusion events. Indeed, in DKD cells, the footprint area increased 2.37+/- 0.28 (n=11) times during stimulation, a change similar to that found in DKD PC12 cells re-expressing Munc18-1 (2.31+/-0.34 (n=9)). TIRFM of DKD PC12 cells co-transfected with NPY-Cherry and Lifeact-GFP revealed that footprint area increase is mainly driven by the extension of F-actin and that NPY-Cherry containing vesicles actively translocate along these new tracks. **Conclusions:** The footprint expansion observed in PC12 cells during stimulated exocytosis relies on the remodeling of the cortical actin network rather than the increase in membrane area resulting from exocytotic fusion events.

POS-WED-074

A DYD MOTIF IN MYOSIN VI SMALL INSERT MEDIATES ACTIVITY-DEPENDENT ANCHORING OF SECRETORY VESICLES TO F-ACTIN IN NEUROSECRETORY CELLS

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Neurosecretory vesicles (SVs) are mobilized and recruited to the cortical actin network where they are shuttled to the plasma membrane to undergo Ca^{2+}-dependent fusion. The mechanism of such recruitment is currently unknown. **Purpose:** To characterize the mechanisms involved in SVs mobilization in the cortical region, we performed a screening for proteins able to interact with SVs in a Ca^{2+}-dependent manner. **Methods and results:** By using SVs pull down assay coupled to tandem mass spectrometry we identified and confirmed that Myosin VI (MyoVI) interacts with SVs in a Ca^{2+}-dependent manner. Interfering with MyoVI function in PC12 cells, either by overexpressing a tail domain mutant protein or by knock down using shRNA, reduces the density of SVs near the plasma membrane measured by TIRF microscopy (* $p<0.05$). MyoVI knock down cells exhibit a significant reduction (** $p<0.01$) in their ability to release NPY-hPLAP particularly in the second phase of a double stimulation protocol, suggesting a defect in the replenishment of SVs in the cortical region. MyoVI Small Insert expression leads to the potentiation of exocytosis and clustering of SVs to F-actin – an effect relying on a putative Src phosphorylation DYD motif identified by in silico analysis. Indeed, expression of MyoVI Small Insert DYD motif deletion mutant, and/or treatment with specific Src inhibitor does not potentiate NPY-hPLAP evoked release and prevent the activity-dependent recruitment of SVs (n=3). **Conclusion:** Myosin VI Small Insert isoform plays a key role in maintaining the active pool of SVs near the plasma membrane by recruiting SVs to the cortical actin network in an activity-dependent manner.

POS-WED-075

DHA SUPPLEMENTATION PREVENTS THE INCREASE OF CANNABINOID-1 RECEPTOR IN SPECIFIC BRAIN AREA OF RATS FED A HIGH-FAT DIET

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Purpose: The endocannabinoid system plays central roles in the regulation of energy metabolism. Stimulation of the cannabinoid receptor 1 (CB1R) promotes lipogenesis, increases feeding and enhances reward aspects of eating. Dietary fibres and docosahexaenoic acid (DHA) are suggested to have beneficial effects on lipid metabolism and appetite regulation. However, it is not clear whether the dietary intervention of dietary fibres and DHA are involved in the regulation of CB1R expression in rats fed high-fat (HF) diet. This study investigated the CB1R density in the brain of HF fed rats with supplement of galacto-oligosaccharide (GOS), resistant starch (RS) and DHA. **Methods:** Twenty-five male Wistar rats were randomly divided into 5 groups fed with low-fat diet (LF), HF, and HF supplemented with 5.7% GOS, 5.7% RS and 0.5% DHA for 4 weeks, respectively. The CB1R binding density was examined by [3H]-CP55940 autoradiography. **Results:** The CB1R binding densities were significantly increased in the hypothalamic arcuate nucleus (Arc, 64%, $p<0.001$), substantia nigra (SN, 37%, $p<0.001$) and dentate gyrus (DG, 55%, $p<0.001$) and ventral tegmental area (VTA, 18%, $p<0.05$) of HF-fed rats compared to the LF control. Interestingly, adding 0.5% DHA and RS prevented the increase of CB1R binding densities in the Arc, SN, DG and VTA in rats on HF diet, while adding GOS prevented the increase of CB1R binding densities in the Arc, SN and VTA. **Conclusion:** This study showed that HF diet can increase CB1R binding densities in specific brain regions, and many of these changes can be prevented by adding DHA, GOS or RS in the diet.

POS-WED-076

INTERLEUKIN-6 REGULATION OF GENE EXPRESSION IN BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS

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Purpose: The adrenal medulla assists in coordinating the stress response through various neuronal, endocrine and paracrine signals. Evidence indicates the adrenal medulla also responds to stimuli that originate from activated immune cells including interleukin-6 (IL-6), interleukin-1 and tumour necrosis factor-α, thus forming part of a bi-directional relationship believed to exist between the immune and neuroendocrine systems. We have shown bovine adrenal medullary chromaffin cells to be directly responsive to IL-6. Acute exposure to this cytokine (5-60 min) selectively activates the signal transduction and activator of transcription 3 (STAT-3) and extracellular regulated kinase (ERK1/2) pathways. The current study aims to examine the effects of IL-6 on chromaffin cell gene expression. **Methods:** Chromaffin cell cultures were obtained from bovine adrenal glands through protease and collagenase digestion. The cells were exposed to IL-6 (10 nM for 24h) and the total RNA was extracted and analysed using a bovine-specific microarray (Affymetrix). The expression of selected genes was subsequently verified using qRT-PCR. **Results:** IL-6 increased the expression of around 300 identified transcripts by two-fold or greater. Interestingly, the mRNA for a number of neuropeptides (including galanin, vasointestinal polypeptide, gastrin releasing peptide and parathyroid hormone related peptide was notably increased. Responses in the latter two genes were particularly prominent, with both demonstrating approximately four-fold increases. **Conclusion:** These data provide evidence that chromaffin cells are responsive to IL-6 and that this interaction may alter their neuropeptide synthesis profile. This is important because while the full physiological significance of adrenal medullary neuropeptides is unknown some, including galanin and vasointestinal polypeptide, may act via the adrenal cortex to limit the inappropriate escalation of an inflammatory response.

POS-WED-077

OLANZAPINE TREATMENT AND TIME-DEPENDENT CHANGES OF HYPOTHALAMIC AMPK-ACC-CPT1 SIGNALLING, FOOD INTAKE AND BODY WEIGHT IN RATS

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Olanzapine, a widely used second generation antipsychotic drug (SGA), has a high risk of inducing hyperphagia and weight gain. Acute olanzapine treatment has been reported to increase hypothalamic phospho-AMP-activated protein kinase (pAMPK) level. The activation of hypothalamic AMPK-Acetyl-CoA carboxylase (ACC)-carnitine palmitoyltransferase 1 (CPT1) pathway largely contributes to excessive food intake and weight gain. **Aim:** This study aimed to investigate the effects of short- (8 days), middle- (15 days) and long-term (36 days) treatment of olanzapine on hypothalamic AMPK-CPT1 signalling. **Methods:** Female Sprague-Dawley rats were treated orally with olanzapine (1 mg/kg, t.i.d., n=12/group) or vehicle for 8, 15 and 36 days. The expression of hypothalamic AMPK, pAMPK, ACC, phospho-ACC (pACC) and CPT1c were detected by Western blot. **Results:** Olanzapine significantly increased daily food intake on days 4-8 ($P<0.001$) and weight gain after short- ($p<0.001$) and middle-term ($p<0.01$) treatment. The long-term treatment of olanzapine maintained high level of body weight through experimental period ($p<0.05$), however daily food intake did not differ from controls during days 14-36 of the treatment. Olanzapine tended to up-regulate the hypothalamic pAMPK and CPT1c levels ($p=0.10$, $p=0.08$, respectively) after short-term treatment. Long-term treatment however significantly decreased the levels of hypothalamic pAMPK and pACC ($p<0.01$). **Conclusion:** The results confirmed the report of food intake and body weight change induced by olanzapine in previous studies, and we suggest a time-dependent change in the hypothalamic AMPK-ACC-CPT1 signalling during olanzapine treatment.

POS-WED-078

GINSENOSIDE RB1 IMPROVES CENTRAL LEPTIN SENSITIVITY IN HIGH-FAT DIET-INDUCED OBESE MICE

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Purpose: Leptin inhibits feeding and increases energy expenditure through its central action, especially in the hypothalamus. Central leptin resistance is a key feature of obesity and its related metabolic disorders. Triterpene saponins (TS) can reduce food intake and body weight. Little is known if their beneficial effect is via improving central leptin sensitivity. In this study we tested the central leptin sensitivity of several TS, including ginsenoside Rb1 (GS), astragaloside II (AS) and isoastragaloside I (IAS). **Methods:** Study 1: Four groups of male mice (n=12) on low-fat diet (LF) were treated with GS, AS, IAS and vehicle (i.p. daily for two days), then followed by leptin or saline intracerebroventricular (i.c.v.) injection. Study 2: after high-fat diet (HF) for eight weeks, two groups of male mice (n=12) were treated with GS and vehicle (i.p. daily for two days) then followed by leptin or saline i.c.v. injection. The food intake was measured at 1, 4 and 24 hours after i.c.v. injection. **Results:** On LF diet, after i.c.v. leptin injection, mice treated with GS had significantly decreased food intake than controls for 1 hour (-50%, $p=0.026$); however the AS and IAS treatments did not show such effect as GS. After HF diet for 8 weeks, central leptin sensitivity was blunted. Importantly, GS treatment reinstalled leptin sensitivity as seeing a significant decrease in food intake after i.c.v. leptin injection for 24 hours (-30%, $p=0.017$). **Conclusion:** This study suggested that ginsenoside Rb1 enhances leptin sensitivity and corrects leptin resistance in high-fat diet-induced obese mice.

POS-WED-079

DIFFERENTIAL PRODUCTION OF ENDOGENOUS CANNABINOIDS AND PROSTAGLANDINS DURING DIFFERENT MATING STRATEGIES IN THE RAT

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Purpose: The endogenous cannabinoids (eCBs), anandamide (AEA) and 2-arachidonyl glycerol (2-AG), are involved in reproduction. Related lipid signaling molecules, the prostaglandins, have also been implicated in reproduction with proposed roles in sexual differentiation of the brain during development and the initiation of ovulation in the hypothalamus. To date, work has focused on lipid signaling that regulates brain areas that play a regulatory and preparatory role in the development and maintenance of reproductive behaviors. Here, we test the hypothesis that mating behaviors directly result in the production of lipid ligands, and that specific mating strategies differentially regulate lipid production in brain. **Methods:** Lipid analysis using liquid chromatography/tandem mass spectroscopy was performed on brains from female rats in proestrus (n=6/group) during two different mating strategies (pace mated, standard mated) and compared with two control groups (chamber exposed, not tested). eCBs and related lipids (9 different lipids) were measured in 8 brain regions (olfactory bulb, hypothalamus, hippocampus, thalamus, striatum, midbrain, cerebellum, and brainstem). **Results:** Prostaglandin (PGE2 and PGF2α) levels increased by 100% ($P<0.05$) in the hypothalamus during paced mating, potentially indicating a role in mating-induced ovulation. Brainstem levels of both AEA and 2-AG significantly decreased after paced mating (approximately 25%, $P<0.05$); potentially reflecting a role in sex-induced analgesia. More modest changes in both lipid classes were observed in other regions. **Conclusions:** These data provide a novel framework to investigate neuroendocrine and perceptual changes in the brain in response to different mating strategies in the rat.

POS-WED-080

GABA RECEPTOR POLYMORPHISM, FUN SEEKING AND HEROIN DEPENDENCE

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Purpose: Heroin abuse is considered to be multifactorial, with the genetic, personality and environmental factors interacting in a complex manner from initial predisposition to the development and maintenance of dependence. Objective of this study is to investigate the role of GABA receptor polymorphisms in relation to heroin dependence. **Methods:** In this study, we investigated 6 single-nucleotide polymorphisms (SNPs) of the GABAA receptor subunit gene cluster in chromosome 5q33 in 157 Sinhalese male dependent heroin users and 157 matched controls. **Results:** After controlling for multiple testing using the permutation approach, we found GABAAα6, rs3219151, ($P = 0.03$) and GABAAγ2, rs211013, ($P = 0.003$) were significantly associated with dependent heroin use. SNPs rs211013 and rs211014 of GABAAγ2 were in strong linkage disequilibrium. Two haplotypes of these 2 SNPs, C-A ($P = 0.0001$) and C-G ($P = 0.002$), were also significantly associated with dependent heroin use. Further, rs211013 was also associated with the fun-seeking personality trait. Mediation analysis revealed a significant indirect effect of rs211013 via fun-seeking personality on risk of heroin dependence. **Conclusion:** The present study confirmed the role of GABAA receptors in predisposition to heroin dependence. This effect is likely to be mediated, at least in part, by the fun-seeking personality trait.

POS-WED-081

EPO AMELIORATES AXONAL DAMAGE, ATTENUATES MACROPHAGE INFILTRATION AND RESTORES MOTOR FUNCTION IN A COMBINED MODEL OF TAI AND HYPOXIA

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Purpose: Diffuse brain injury is the predominant form of TBI in humans, which when associated with hypoxia worsens neurological outcomes. Here we seek to determine the ability of erythropoietin (EPO) to ameliorate axonal damage and neuroinflammation, and improve outcome in a rat model of diffuse TBI and hypoxia, and elucidate its mechanisms of action. EPO confers neuroprotection in focal TBI, however EPO has not been investigated in a diffuse TBI-hypoxia paradigm. **Methods:** Diffuse traumatic axonal injury (TAI) was produced by dropping a 450g weight from a height of 2m, and hypoxia by ventilation with 14%O2 for 30min after TAI. Rats were administered rhEPO-alpha (5000IU/kg, i.p.) or vehicle at 1 and 24h after injury. Motor function was assessed using the Rotarod up to 14d. Immunohistochemistry was performed for axonal pathology (neurofilament 200kDa), dendrite integrity (MAP2), and macrophage/microglial infiltration (CD68) at 1, 7 and 14 d. **Results:** In TAI+hypoxia rats, EPO lead to improvement on the Rotarod by 5d compared with TAI+hypoxia+vehicle ($p<0.05$). Neurofilament staining revealed extensive damage in the corpus callosum and brainstem of TAI+hypoxia+vehicle rats, whereas in TAI+hypoxia+EPO rats, axonal pathology was reduced at 1d and 7d ($p<0.05$). CD68+ macrophages were observed at 7d and 14d in TAI+hypoxia+vehicle, with a decrease in TAI+hypoxia+EPO rats at both 7d and 14d post injury. Loss of MAP2 was observed in the cortex and caudate of TAI+hypoxia+vehicle rats, whilst EPO treated rats retained MAP2 integrity ($p<0.05$). **Conclusion:** These results demonstrate that in rats subjected to TAI+hypoxia, EPO improves motor function; which is likely due to reduced axonal pathology, attenuated macrophage infiltration and retained dendritic integrity.

POS-WED-082

A CELL CULTURE MODEL FOR INVESTIGATING MICROGLIAL RESPONSES TO FOCAL CELL DEATH

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Purpose: Most currently available cell culture models do not allow investigation of glial cell responses to a region of localized cell death, as commonly occurs in stroke and trauma. We have adapted a published technique for inducing focal cell death in mixed glial cultures to investigate peri-lesional microglial responses. **Methods:** Confluent primary cortical cultures grown on poly-L-lysine coated coverslips were exposed to highly localized cooling by applying the end of a liquid-nitrogen-cooled copper rod (10mm diameter) to the base of the culture dish for 6-7 seconds. Cells were fixed for analysis up to 48 hours post-injury. **Results:** The treatment rapidly produced a well-defined circular central region of near complete cell death while cells outside this area were spared. Initial studies using cultures grown under standard culture conditions demonstrated that the injury induced microglial proliferation with a 1.96-fold increase in numbers between 2 and 48 hours post-lesion ($n=4$, $p<0.001$). Culture conditions were subsequently modified to increase the proportion of microglia exhibiting a ramified morphology associated with a basal “surveillance” state and decrease those with an amoeboid morphology typically seen with cell activation. In these cultures, cooling produced essentially complete loss of ramified microglia immediately surrounding the region of cell death with a marked increase (from 42% to 85%, $n=3$, $p<0.001$) in amoeboid cells by 2 hours, indicative of rapid microglial activation. Similar but smaller changes were seen in regions further from the site of injury. **Conclusion:** This focal cooling model recapitulates key features of microglial responses to focal injury *in vivo*. It can be used to further investigate microglial activation and microglia-astrocyte interactions induced by focal injury.

POS-WED-083

METHIONINE OXIDATION INDUCES APOLIPOPROTEIN-D AGGREGATION

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Purpose: Selective oxidation of methionine (Met) residues may induce protein aggregation. We have shown that apolipoprotein D (apoD) acts as a lipid antioxidant via an interaction of Met93 with lipid hydroperoxides (LOOH). In this reaction L-OOH are reduced to non-reactive lipid hydroxides (LOH) and Met93 is oxidised to Met sulfoxide (MetSO). We examined the impact of apoD Met oxidation on aggregation and also assessed Alzheimer’s disease (AD) brain for evidence of apoD aggregates. **Methods:** ApoD and Met to Ala mutants in which either one (apoD-M49A, apoD-M93A, apoD-M157A) or all (apoD-MA) Met residues were replaced by Ala were incubated with LOOH for up to 8 hours. Oxidation of apoD Met residues was confirmed by HPLC and amino acid analysis. Protein was ethanol precipitated from the reaction mixture and analysed using SDS PAGE and western blotting to assess aggregation. Insoluble protein fractions were also isolated from AD brain tissues (Sydney Brain Bank, Prof Glenda Halliday) using guanidine hydrochloride extraction and similarly analysed SDS PAGE and western blotting. **Results:** Incubation of apoD with LOOH resulted in selective oxidation of Met93. Oxidation of Met93 increased aggregation of wild type (wt) apoD in a time-dependent manner. Compared to wt apoD, apoD-MA and apoD-M93A mutants were resistant to LOOH-induced aggregation. The predominant apoD aggregate formed was a 50 kDa dimer. Increased apoD dimerization was also clearly detected in AD hippocampus but not in matched control tissues. **Conclusions:** Selective oxidation of apoD Met93 by LOOH induces protein aggregate formation. ApoD aggregates that may result from LOOH interactions are also detected in AD hippocampus.

POS-WED-084

FORMALDEHYDE INDUCES TAU HYPERPHOSPHORYLATION IN N2A CELLS AND MOUSE BRAINS

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Purpose Formaldehyde exposure induces human cognitive impairment and animal memory loss. The level of endogenous formaldehyde is abnormally elevated in brains of Alzheimer’s disease (AD) animal models and patients when their memory starts to decline. Considering the characteristics of hyperphosphorylated Tau observed in neurodegeneration, it is important to study the relationship between formaldehyde and Tau hyperphosphorylation to clarify the mechanism of formaldehyde-induced tauopathy. **Methods** N2a Cells were treated with formaldehyde at 0.5 mM, then harvested for western blotting or cytoimmunofluorescence at 2, 4, 8, 12, 24 hours, cell viability were also measured. C57BL/6 mice were injected with formaldehyde through tail vein, and after 2, 12, 24, 48, 72, 168 hours, sacrificed for western blotting and histochemistry. Cell nuclei are separated from cytoplasm by Cytoplasmic and Nuclear Protein Extraction Kit to see distribution of phosphorylated Tau. Anti-phosphorylated Tau antibodies were used (anti-pS396 and T181). **Results** Western blotting results show that formaldehyde treatment leads to the accumulation of GSK-3β in the nucleus and to high levels of both cytoplasmic and nuclear Tau phosphorylation in N2a cells and mouse brains. Inhibition of GSK-3β decreases the phosphorylation level of Tau in N2a cells. Immunohistochemistry images also show that Tau phosphorylation increases under the treatment of formaldehyde in both N2a cells and mouse **brains, especially the nuclear Tau. Conclusion**: Formaldehyde induced Tau hyperphosphorylation in both cytoplasmic and nucleus in N2a cells and mouse brains. This not only reveals formaldehyde as an inducer of protein hyperphosphorylation, but also suggests a new mechanism underlying tauopathies.

POS-WED-085

INCREASES IN INTRACELLULAR CLUSTERIN RELATE TO TAU PATHOLOGY IN ALZHEIMER'S DISEASE

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Purpose: Genome-wide association studies have identified clusterin (CLU) as a risk factor for Alzheimer's disease (AD). This protein is processed into three alloforms, cytoplasmic (cCLU), nuclear (nCLU) and an excreted soluble form (sCLU). The aim of this study was to quantify the protein levels of these alloforms and determine their association with AD pathologies. **Methods:** Brain tissue from 6 AD patients, 6 prodromal AD, and 6 controls was obtained from the Sydney Brain Bank and NSW TRC. Analysis of sCLU, cCLU, nCLU, Aβ40 and Aβ42 (soluble, membrane-bound and insoluble), tau (soluble and hyperphosphorylated) and ApoE was carried out by Western blot and ELISA. CLU immunohistochemistry was also performed. **Results:** The data showed a decrease in sCLU in prodromal AD compared to controls (p=.006) and AD (p=.004), suggesting an early reduction in sCLU prior to dementia onset. Levels of sCLU were negatively associated with ApoE (-.59 p=.01). In determining associations with AD pathologies, our data demonstrated a negative correlation between ApoE and soluble (-.645, p=.04) and SDS (-.66, p=.04) Aβ40, although no relationship between sCLU and Aβ was observed. Analysis of intracellular CLU (cCLU and nCLU) revealed a significant increase in AD compared to controls (p=.004) and prodromal AD (p=.008); a finding confirmed by immunohistochemistry. Intracellular CLU levels also showed an inverse association with soluble tau (-.67, p=.045) and a positive correlation with insoluble, hyperphosphorylated tau (.74, p=.02). **Conclusion:** This study supports differential roles for secretory and intracellular alloforms of CLU in the onset and progression of AD with intracellular forms playing a potentially important role in the neuronal tau pathology that underlies the disease process.

POS-WED-086

EFFECTS OF DOPAMINE D_2 RECEPTOR PARTIAL AGONIST BIFEPRUNOX ON BODY WEIGHT GAIN, FOOD AND WATER INTAKE IN MALE RATS

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Purpose: Whilst successful in the treatment of the symptoms of schizophrenia, atypical antipsychotic drugs have been found clinically to induce serious metabolic side effects, including body weight gain/obesity. Numerous studies have found a relationship between dopamine D_2 activity and feeding behaviour. This study aimed to investigate the effects of antipsychotic drugs haloperidol (D_2 antagonist), aripiprazole (a D_2 partial agonist and a functional selective ligand at D_2 receptors), and bifeprunox (a D_2 partial agonist) on body weight gain, food and water intake. **Methods:** Male Sprague-Dawley rats (n=6/group) were administered orally with either aripiprazole (0.75mg/kg, t.i.d.), haloperidol (0.1mg/kg, t.i.d.), bifeprunox (0.8mg/kg, t.i.d.), or vehicle (control), for 10 weeks. **Results:** Rats treated chronically with bifeprunox exhibited significantly reduced body weight gain (BWG), food intake (FI) (both $p<0.05$) and water intake (WI) ($p<0.01$) compared to the control. Significant differences were found from treatment week 1 (WI; $p<0.01$) or week 6 (BWG and FI; $p<0.05$) onwards. However, aripiprazole and haloperidol treatment had no effect on any of these parameters upon comparison to the control. **Conclusion:** These findings revealed that only bifeprunox, but not aripiprazole, reduced weight gain, as well as food and water intake in male rats, although both are partial agonists. Differing from bifeprunox, aripiprazole is also a D_2 functionally selective drug, which may explain its less side-effect. These results support the important roles of dopamine D_2 receptors in the regulation of food intake and body weight.

POS-WED-087

TIME-DEPENDANT ALTERATIONS OF HYPOTHALAMIC ENERGY REGULATORY NETWORK BY OLANZAPINE IN RATS

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Olanzapine treatment is associated with increased food intake and weight gain. The orexigenic hormone ghrelin and its receptor (GHSR) are associated with hyperphagia, which may be resulted from the altered energy regulatory network within the hypothalamus involving the pro-opiomelanocortin (POMC). Recent studies have shown that olanzapine treatment was associated with increased hypothalamic GHSR and reduced POMC mRNA levels, but the time-dependent response has not been explored. **PURPOSE:** To examine the time-dependent response of hypothalamic GHSR and POMC levels under olanzapine treatment, and the potential correlations with food intake and body weight gain in a rat model. **METHODS:** Rats were treated with olanzapine (1mg/kg, orally, 3x/day, n=6/group) or vehicle for 1 week (short-term), 2 weeks (medium-term), or 5 weeks (long-term). Food intake, body weight and brown adipose tissue (BAT) temperature were monitored. Hypothalamic levels of GHSR and POMC were examined by Western blot. **RESULTS:** BAT temperature was reduced by olanzapine (45-150 min after administration) in the long-term group only, but not in the short-term or medium-term groups. Olanzapine significantly up-regulate hypothalamic GHSR levels by 23% (short-term), 18% (medium-term) and 28% (long-term) ($p<0.05$), while down-regulating hypothalamic POMC levels by 36% (short-term), 30% (medium-term) and 19% (long-term) ($p<0.05$). The hypothalamus expression of GHSR was positively correlated with cumulative weight gain in short-term ($r=0.82$, $p<0.01$) and medium-term ($r=0.64$, $p<0.05$), and with cumulative food intake in short-term ($r=0.81$, $p<0.01$) and medium-term ($r=0.71$, $p<0.01$). **CONCLUTION:** Olanzapine significantly increased hypothalamic GHSR expression in a time-dependent manner, which may lead to decreased levels of POMC and hence responsible for the increased food intake, in particular at early stage of treatment, and weight gain.

POS-WED-088

ALTERED PERIVASCULAR INNERVATION OF PLANTAR METATARSAL ARTERIES IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT

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Purpose: Impaired sympathetic neurovascular transmission (NVT) may contribute to vascular-related diabetic complications. In streptozotocin (STZ)-induced type I diabetic rats without insulin support NVT was reduced in plantar metatarsal arteries (PMA) which supply skin of the hindpaw digits. This impairment of NVT was not observed in STZ-rats treated with a low dose of insulin (1 unit/day) despite their being severely diabetic (blood glucose >20mM). In both groups, there were changes in perivascular sympathetic innervation. In the present study we analysed the changes in perivascular innervation and assessed the effects of reducing blood glucose levels with a high dose of insulin. **Methods:** PMAs of STZ-treated rats (60mg/kg, i.p.) receiving a low (n= 6) or high (n= 7) dose of insulin for 12 weeks were compared to vehicle controls. Perivascular axons were detected immunohistochemically using antibodies against tyrosine hydroxylase (TH). Changes in TH levels were quantified relative to β-actin by western blotting. The axon plexus was quantified in terms of the number, TH+ brightness and length of fluorescent structures intercepting horizontal lines placed on the images. **Results:** PMAs from diabetic rats receiving a low dose of insulin (terminal blood glucose 27.7±1.5mM) had a reduce frequency, but an increased TH+ brightness and width of intercepts ($p<0.05$) compared to control. Western blotting analysis also revealed an ~2-fold increase in TH expression ($p<0.05$). These changes were not observed in animals receiving a high dose of insulin (terminal blood glucose 9.9±1.9mM). **Conclusion:** The changes observed in the low dose insulin group are most likely due to hyperglycemia. This makes PMAs a suitable model to assess the development and prevention of diabetes-induced sympathetic neuropathy.

POS-WED-089

SUMO-1 CO-LOCALISES WITH A SUBSET OF LYSOSOMES ASSOCIATED WITH GLIAL PROTEIN INCLUSION BODIES

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Many neurodegenerative diseases are characterised by microscopically-visible protein aggregates, or inclusion bodies, within neural cells. The ubiquitin homologue, SUMO-1, has been identified in sub-domains of pathological inclusion bodies in several neurodegenerative diseases. Inclusion bodies are believed to be formed actively in a defensive response to soluble cytotoxic protein aggregates. We hypothesised that SUMO-1 may become associated with lysosomes in this response. Fluorescence Immunohistochemistry of brain tissue from multiple system atrophy and progressive supranuclear palsy cases (n = 10) revealed frequent SUMO-1 sub-domains within and around inclusion bodies with punctate co-localisation of SUMO-1 and a lysosomal marker, Cathepsin D. In inclusion-bearing cells, 75% of lysosomes showed co-localisation with SUMO-1 compared to 20% in inclusion-free cells and 10% in age-matched normal brain tissue. This difference was statistically significant with $P < 0.05$. Cell culture treatments of 1321N1 glioma cells using the proteasome inhibitor MG132 revealed SUMO-1 and lysosomal co-localisation, 48hrs post-treatment. Lysosome isolation and Western blot analysis of the lysosome-rich fraction identified a SUMO-1 +ve 90kDa band that increases in treated cells, 48hrs post-treatment. Furthermore, the 90kDa band was also immunopositive for HSP90. These finding suggest a molecular association between SUMO-1 and autophagy in the response to protein aggregation in gliopathies.

POS-WED-090

REDOX ACTIVITY OF METHIONINE-CONTAINING PEPTIDES DERIVED FROM APOLIPOPROTEIN-D

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Purpose: Recent studies indicate apolipoprotein-D (apoD) acts as a lipid antioxidant in the brain. Research from our laboratory suggests that one of the three apoD Met residues (apoD Met93) is crucial for this lipid antioxidant function. It is not clear how the amino acid sequence surrounding apoD Met residues may affect peptide redox activity and this was addressed in the current study. **Methods:** We synthesised nine peptides based on the three Met residues within apoD and this included Met to Val substitutions to determine the specific redox activity of Met residues. In addition, selenomethionine (SeMet) mutants were also generated in order to establish whether selenium confers a stronger redox activity than sulfur. Peptide interaction with hydrogen peroxide and autoxidised liposomes was then assessed and conversion of Met to Met-sulfoxide was assessed by HPLC methods. **Results:** Our data indicate that Met peptides are redox active and have antioxidant potential. This antioxidant action is lost in the Met to Val mutants and increased ~100 fold in the SeMet mutants. The impact of neighbouring amino acids on Met redox activity and antioxidant function is currently under investigation. **Conclusion:** ApoD Met-containing peptides are redox active and also act as lipid antioxidants. Substitution of selenium for sulfur in the Met side chain dramatically increases peptide redox activity.

POS-WED-091

METABOLIC EFFECTS OF PROPIONYL-COA PRECURSORS IN NEURONAL CELLS *IN VITRO* AND *VIVO*

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Purpose: Anaplerosis, the refilling of the intermediates of the tricarboxylic acid cycle, plays a crucial role in metabolism. It is believed that anaplerosis in the brain occurs mainly through pyruvate carboxlyase, but the contribution of the propionyl-CoA carboxylation pathway has not yet been precisely examined. The triglyceride of heptanoate (C7 fatty acid), triheptanoin, is a tasteless oil was developed as an anaplerotic substrate through this pathway and is used to treat rare metabolic disorders. Mitochondrial beta-oxidation metabolizes heptanoate to propionyl-CoA, which after carboxylation to methyl-malonyl-CoA can produce succinyl-CoA. Heptanoate is also metabolized by the liver to the C5 ketones, beta-ketopentanoate and/or beta-hydroxypentanoate, which are released into the blood and thought to enter the brain via monocarboxylate transporters. Oral triheptanoin has recently been discovered to be reproducibly anticonvulsant in acute and chronic mouse seizure models. **Methods:** Here we investigate alterations of brain and neural metabolism after triheptanoin administration. **Results:** Triheptanoin feeding resulted in increased propionyl- and methyl-malonyl-CoA levels in brains of the chronic epilepsy mouse pilocarpine model (n=10). Also, incubation of C6 glioma cells with [1-^{13}C]glucose in the presence of heptanoate showed decreased 13C labeling of metabolites (n=3), indicating that the propionyl-CoA pathway is anaplerotic in brain cells. Metabolic alterations in mitochondria in neuronal cells in vitro and in vivo are being investigated, including their membrane potential and ATP production. **Conclusion:** This work will contribute to the elucidation of the anticonvulsant mechanism of triheptanoin and its clinical potential for the treatment of epilepsy and other disorders of the brain.

POS-WED-092

EXPRESSION AND REGULATION OF α-SYNUCLEIN SPLICE VARIANTS IN HUMAN ALCOHOLIC BRAIN

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Chronic alcohol misuse results in alterations in gene expression in brain regions susceptible to the neurotoxic effects of alcohol. One such gene, α-synuclein, is a key regulator of dopaminergic neurotransmission. Previous studies by ourselves and others have identified α-syn to be differentially expressed in the brain and blood of alcoholics, whereas other studies have shown no such change. α-Syn exists in a number of different splice variants and its expression is influenced by both genetic factors and microRNAs. Here we investigate the influence of these factors on the expression of α-syn in human alcoholic brain. Real-time PCR was used to quantify α-syn mRNA expression in autopsy samples (26 controls, 26 alcoholics, 12 alcoholic cirrhotics) of human prefrontal cortex using splice variant selective primers for SNCA140, SNCA112 and SNCA115. Alcoholics were classified by having consumed over 80g of ethanol per day for most of their adult lives whereas controls consumed 20g or less per day. The expression of SNCA140 and SNCA112 was significantly lower in the prefrontal cortex of cirrhotic alcoholics ($P = 0.030$ and $P < 0.001$ respectively) whereas the expression of SNCA115 was significantly higher in cirrhotic alcoholics ($P = 0.006$). The expression of wildtype SNCA140 transcripts was influenced by genetic variation in *SNCA*-Rep1; individuals with at least one copy of the 267 bp allele had lower expression levels of SNCA140 and were more likely to exhibit an alcohol misuse phenotype ($OR_{adj} = 2.21$, $df = 1$, $P = 0.012$). Furthermore, the expression of all three splice variants was regulated by miR-153 and miR-7, the expression of which is known to be up-regulated in the prefrontal cortex of alcoholics.

POS-WED-093

BRAIN STRUCTURAL AND FUNCTIONAL CHANGES IN A MOUSE MODEL OF FOETAL ALCOHOL SYNDROME

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Prenatal alcohol exposure can result in patterns of physical and mental abnormalities described as fetal alcohol spectrum disorder (FASD) in which the most severe form is fetal alcohol syndrome (FAS). The clinical features of FAS/FASD include growth restriction, with or without craniofacial abnormalities, and brain structural and functional abnormalities. The adverse outcomes of prenatal alcohol exposure are linked to exposure timing and dosage, and symptoms can vary from case to case. This makes clinical feature-based diagnosis much harder. Epigenetics is considered to play a role in the etiology of FAS/FASD, as DNA methylation, post-translational histone modifications and microRNA expression patterns have all been reported to be altered in response to alcohol exposure in animal models. Such epigenetic changes could be used as biomarkers to enable early and accurate diagnosis. **Purpose**: In this study, a mouse model of moderate prenatal alcohol exposure, established previously, is further characterized to identify human FAS/FASD-like phenotypes. **Results**: The peak maternal blood alcohol concentration measured is 96mg/dl, and the exposure period is from gestational day 0.5 to 8.5. This model mimics chronic, moderate drinking behavior during the first 3 weeks of pregnancy in humans. Magnetic resonance imaging-based structural analyses of the brain revealed a significant reduction of relative hippocampal and caudate putamen volume compared to whole brain volume in the ethanol-exposed group compared to controls (P<0.05). This is reminiscent of changes seen in human FAS/FASD. Moreover, significant behavioural changes are observed in the treatment group across three independent Cohorts (P<0.05). Microarray analyses of various brain regions revealed a number of differentially expressed genes (> 1.5 fold change) in the treatment group compared to controls, and current experiments are examining the epigenetic control of these genes. The possible relationships between the ethanol-induced molecular and phenotypic changes and their contribution to the etiology of FAS will be discussed.

POS-WED-094

CHRONIC INTERMITTENT INHALANT EXPOSURE DURING ADOLESCENCE IN RODENTS RESULTS IN WHITE MATTER ABNORMALITIES: POTENTIAL FOR RECOVERY FOLLOWING ABSTINENCE

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Purpose: The purposeful abuse of inhalants which include toluene is prevalent in adolescent populations, posing a significant risk to the maturing brain, especially white matter tracts. Despite this our understanding of long-term neuropathological implications of toluene abuse is relatively limited. **Methods**: Male adolescent Wistar rats were exposed to either air (n=6) or chronic intermittent toluene (CIT, 3000ppm, n=6) for 3x1hr sessions/week for 8 weeks followed by 8 weeks abstinence. Longitudinal neuropathological changes were measured using MRI (0, 4, 8 weeks after exposure and 8 weeks abstinence) in the anterior commissure and corpus callosum (genu, body and splenium). **Results:** While there was no difference in the volume of white matter tracts (p>0.05), in animals exposed to CIT there was a significant decrease (p<0.05) in axial (AD) and radial diffusivity at 8 (AD; air: 1.446±0.060x10^{-3} mm^2/s vs CIT: 1.124±0.048x10^{-3} mm2/s, p<0.02), but not 4 weeks (AD; air: 1.147±0.022x10^{-3} mm^2/s vs CIT: 1.217±0.044x10^{-3} mm^2/s), in the anterior commissure, but no differences in the corpus callosum (AD in the genu at 8 weeks; air: 1.200±0.031x10^{-3} mm^2/s vs CIT: 1.113±0.049x10^{-3} mm^2/s), when compared to air controls. Differences were no longer apparent after 8 weeks of abstinence. **Conclusions:** Adolescent CIT exposure for up to 8 weeks is sufficient to induce region specific abnormalities to white matter structures. There appears to be a critical period of exposure before these deficits manifest. Importantly there appears to be the potential for recovery following a period of abstinence.

POS-WED-095

DEVELOPMENT OF NOVEL ANTIPSYCHOTIC DRUGS WITH LOWER OBESOGENIC EFFECT

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Background: Olanzapine is an atypical antipsychotic drug with high clinical efficacy, but which causes severe weight gain and metabolic syndromes. It is believed that blockade of the H_1 receptors plays a crucial part in olanzapine inducing weight gain. An antipsychotic with similar binding profiles as olanzapine but with lower affinity for the H_1 receptors may represent a significant advancement in schizophrenia treatment. **Aims:** We have previously reported the *in vitro* pharmacological evaluation of novel olanzapine derivatives (Olz-1 and Olz-2) with thienobenzodiazepine structure. In this study we examined the effect of these compounds on the weight gain. **Methods:** Female Sprague–Dawley rats were treated orally, three times daily with olanzapine, Olz-1 or Olz-2 (3 or 6 mg/kg/day) self-administered in a sweet cookie dough pellet at eight-hourly intervals or vehicle (n = 8/group) for five weeks. Body weight and food intake were monitored every 48 hours. Visceral white fat pads and sub scapular brown fat pads were weighted. **Results:** Olanzapine (3 mg/kg/day and 6 mg/kg/day) significantly increased the total body weight gain compared to controls after five weeks treatment ($F_{2,48}$= 19.008, p < 0.01). Weight gain effect of Olz-1 (-27%, p < 0.01) and Olz-2 (-35%, p < 0.01) was significantly lower than olanzapine groups. Olanzapine (6 mg/kg/day) increased food intake and feeding efficiency. Olz-1 and Olz-2 did not show any significant effect on body weight gain and food intake. Visceral fat mass increased (6 mg/kg/day) in rats treated with olanzapine. The effect of Olz-1 and Olz-2 treatment on adiposity was not significant. **Conclusion:** We suggested that Olz-1 and Olz-2 with lower obesity side effects may advance schizophrenia treatment.

POS-WED-096

EVALUATION OF INFLAMMATORY CYTOKINES IN FRONTOTEMPORAL LOBAR DEGENERATION

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PurposeInflammation is a widely accepted pathological characteristic of many neurodegenerative diseases. Alzheimers disease (AD) and Frontotemporal Lobar Degeneration (FTLD) are two neurodegenerative dementias known to involve pathological protein aggregation and inflammation. Recently our lab has described novel increases in complement and immunoglobulin proteins in FTLD tissue. Differences were observed between the tau-positive and tau-negative subtypes of FTLD which suggest different underlying immune processes. To test this further, differences in inflammatory cytokine levels between these FTLD subtypes were evaluated.**Methods** Tissue was obtained from the Sydney Brain Bank: normal controls (n=11), FTLD (n=13) and AD (n=9). The inflammatory cytokines IL4, IL6, IL8, IL12, IL15, MCP-1 and TNF-alpha were quantified in fresh frozen brain tissue of the superior temporal gyrus via Multiplex ELISA. Cytokines found to be elevated in the ELISA were evaluated using immunohistochemistry on formalin fixed paraffin embedded tissue and quantified. **Results** MCP-1 was found to be increased in all disease groups, particularly in tau-positive FTLD cases (p<0.01). Elevated IL-8 on ELISA was evident in AD cases (p<0.05). Immunohistochemical evaluation showed greater immunoreactivity among neurons in the tau-positive form of FTLD (p<0.001). Differences in both MCP-1 and IL-8 were observed between subtypes of FTLD and AD. (p<0.05). **Conclusion**These findings indicate that inflammation not only differs between tau-positive and tau-negative FTLD cases, but also between tau-positive neurodegenerative dementias. It has also been shown that the pro-inflammatory chemokines MCP-1 and IL-8 are involved in the pathology of these dementias. This further suggests that mechanisms independent of aggregated, hyperphosphorylated tau influence inflammatory processes in these dementias.

POS-WED-097

AGE-RELATED INCREASE IN MITOCHONDRIAL DNA COPY NUMBER IN MIDBRAIN DOPAMINE NEURONS OF THE RAT

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Purpose: Ageing can cause deleterious changes in brain function. One mechanism thought to contribute to these age-related changes is mitochondrial dysfunction. A proposed cause of this dysfunction is mitochondrial DNA (mtDNA) mutation. Mitochondria are vital to numerous cellular processes but are mostly recognized for their energy-producing role, a particularly important role in the nervous system given its high energy demands. MtDNA, contained within the mitochondrial organelles, is present in multiple copies and encodes essential components of the energy-producing mitochondrial respiratory chain. Changes in mtDNA copy number are associated with detrimental effects on mitochondrial, and thus cellular, function. However, the effect of ageing on mtDNA copy number in specific cell types of the CNS is currently unknown. Therefore, the aim of the present study was to characterize the effect of ageing on mtDNA copy number in neuronal and glial cell types in the rat brain. **Methods:** Midbrain dopamine neurons (n=300) from three young adult (4-6 months) and three old (23-26 months) male Fisher 344 rats, were microdissected and pooled for each animal. DNA was extracted and qPCR analysis carried out to allow comparison of mtDNA copy number for the two age groups. **Results:** MtDNA copy number increased from approximately 854 (±49) per cell in dopamine neurons of young animals to approximately 2295 (±260.) in old animals ($p<0.05$). **Conclusions:** These data indicate changes in mtDNA copy number may contribute to age-related changes in dopamine neuron function.

POSWED-098

TESTING NEUROPROTECTIVE AND REGENERATIVE STRATEGIES FOR PARKINSON'S DISEASE IN A ROTENONE RODENT MODEL

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Rotenone, infused into the medial forebrain bundle causes neuronal loss through chronic oxidative stress while surviving neurons show histopathology similar to human Parkinson's disease[1]. This study uses the rotenone model to examine the neuroprotective and regenerative potential of vascular endothelial growth factor (VEGF) and platelet derived neurotrophic factor (PDGF) in combination, known to modulate the glial response and provide neuroprotection in an acute stab injury model[2]. Mature male Sprague-Dawley rats (n=16) were implanted intra-striataly with an infusion cannulae connected to osmotic minipumps (15 ng/day) before receiving a medial forebrain bundle infusion of rotenone 7 days later. Animals were exposed for seven days after lesion to 5-ethynyl-2'-deoxyuridine (EdU) to label proliferative cells. Animals were sacrificed 14 days after minipump implantation. Treatment provided neuroprotection to dopaminergic projections ($p\leq0.05$) concurrently with an increase in vasculature ($p\leq0.05$) in the striatum, demonstrated using immunofloresence. A trend towards an increase in astrocytes and microglia was apparent. Cell proliferation was not detected in the striatum after treatment. However, treatment caused a highly proliferative and vascularised tissue mass ventral to the substantia nigra. The present experiment suggests that a sustained low-dose infusion of VEGF and PDGF may provide neuroprotection, increase vascularisation and the glial response in the striatum after a focal rotenone lesion, suggesting a multifactorial neuroprotective action involving both direct and indirect mechanisms. 1. Norazit, et al (2010) Brain Research, 1360: 119-121. 2. Norazit, et al (2011) Neuroscience 192, 652-660.

POS-WED-099

EFFECT OF TYROSINE HYDROXYLASE ON ALPHA-SYNUCLEIN AGGREGATION

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Parkinson's disease (PD) is a common neurodegenerative disorder. One of the major pathological features of PD is the development of intracytoplasmic inclusions called Lewy bodies. α-synuclein (α-Syn) is a major component of Lewy bodies. **Purpose:** To investigate the effect of tyrosine hydroxylase (TH) on α-Syn aggregation. **Methods:** Recombinant TH and α-Syn were expressed in *E-coli* and the proteins were purified. Their interaction was examined by co-incubation at 37°C for up to 18 hours. **Results:** TH induced the formation of an SDS resistant α-Syn multimer of 90-100 kDa. This complex was only found in the pellet after centrifugation at 18,000 X g for 20min at 4°C (n=4). Incubation of α-Syn with a series of control proteins did not generate the 90-100 kDa α-Syn complex (n=3), indicating that it was a specific effect of TH. The formation of this α-Syn complex was found to be TH concentration and time dependent (up to 18 hours). The 90-100 kDa α-Syn complex could not be dissociated by urea (8M) or guanidine hydrochloride (5M) (n=4). Aggregates containing the 90-100 kDa α-Syn complex could be solubilised by guanidine hydrochloride, but not by urea. **Conclusions:** This data suggests that the aggregation of α-Syn is influenced by the presence of TH and therefore the aggregation process in TH containing neurons may be different from that in other neurons.

POS-WED-100

ARE THERE VULNERABILITY FACTORS FOR THE SELECTIVE LOSS OF VENTRAL DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA IN PARKINSON'S DISEASE?

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Background: Selective degeneration of dopamine neurons in the ventral tier of the substantia nigra (SN), rather than dorsal tier and ventral tegmental area (VTA), occurs in Parkinson's disease (PD) [1]. This differential cell death may result from differences between cells in their protein expression [1]. Previous rodent studies suggest that the deleted in colorectal cancer (DCC) receptor and PITX3 may be expressed only in the vulnerable ventral SN cells [1] and orthodenticle homeobox 2 (OTX2) only in the resistant VTA cells, implicating these proteins in differential cell vulnerability. **Purpose:** To determine whether expression of these proteins differentiates neurons in ventral and dorsal SN tiers. **Methods:** Formalin-fixed midbrains from five healthy individuals (aged 81.4±2.3) without significant neuropathology were obtained from the Sydney Brain Bank following study approvals. DCC, PITX3 and OTX2 expression was investigated using single immunoperoxidase and double immunofluorescence (with tyrosine hydroxylase, TH; identifies dopaminergic neurons) in serial transverse sections of the entire SN. **Results:** DCC and PITX3 were expressed in the majority of SN neurons (~96±3% of TH-immunoreactive neurons). Expression of these proteins was seen in both the ventral and dorsal tiers throughout the rostrocaudal extent of the SN. OTX2 was not present in the TH-immunoreactive SN or VTA neurons. **Conclusion:** These data demonstrate that these proteins are not differentially distributed in different populations of dopaminergic neurons in humans, and thus their expression is unlikely to underlie the differential vulnerability within the SN observed in PD.

POS-WED-101

SCREENING NFE2L2 FOR RARE VARIANTS IN PARKINSON'S DISEASE PATIENT-DERIVED CELL LINES

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Purpose: Impairment of antioxidant defence mechanisms and oxidative stress has been implicated in both genetic and sporadic cases of PD. Transcriptomic analyses of patient and control-derived hONS cell lines identified the NRF2-mediated antioxidant response pathway as the most differentially expressed between patients and controls. Functional experiments using these cell lines have further demonstrated reductions in associated metabolic function, including reduced glutathione levels and MTS, suggesting deficiencies in NRF2 function. We hypothesised that rare sequence variants within the coding regions of the NRF2 gene (*NFE2L2*) may underlie changes in NRF2 function. We therefore decided to screen our hONS cell lines for rare variants in the coding region of *NFE2L2*. **Methods:** hONS cell lines were established from PD patients (n=33) and unaffected controls (n=31). Genomic DNA extracted using standard methods. The exons of *NFE2L2* were screened using high resolution melt (HRM) analysis. Sensitivity was tested using control assays for three polymorphisms found at the *NFE2L2* genomic locus (rs6726395, rs2364722 and rs2364725). Melt curves that deviated from those of reference samples were sequenced to confirm and identify possible causative variants. **Results:** HRM analysis was sensitive enough to accurately detect three known polymorphisms. These SNPs are associated with PD in our cell lines. No novel *NFE2L2* sequence variants were detected using this HRM analyses. **Conclusions:** Overall, we determined that HRM analysis is a sufficiently sensitive tool for use in genetic screening. However, our findings suggest that rare sequence variants in the *NFE2L2* gene are not responsible for the functional deficits seen in our patient cell lines.

POS-WED-102

EXPRESSION OF ABCA5 IS ALTERED IN PARKINSON'S DISEASE BRAIN

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Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting from degeneration of dopaminergic neurons impacting on muscles and movement. The pathological hallmark of PD is the presence of aggregated alpha-synuclein associated with lipids. Lipids are transported around the brain by a group of proteins called ATP-Binding Cassette subfamily A (ABCA) transporters and in recent years there has been mounting evidence indicating that ABCA transporters regulate a number of neurodegenerative disease processes. However, the role of ABCA transporters in the pathological processes of PD is fundamentally unknown despite the fact that alpha-synuclein pathology is intrinsically associated with lipids. Only recently a genome-wide association study reported that ABCA5 was genetically associated with a reduced risk for PD. In this study we investigate whether the expression of ABCA5 is altered in PD brain. We also investigate the impact of the plasma membrane lipid sphingomyelin on ABCA5 and alpha-synuclein expression. The expression of ABCA5 (as measured by qRT-PCR) in PD brains (amygdala, n=10) was significantly elevated compared to the control brains ($p<0.05$), possibly as a protective response to the disease. We also treated SK-N-SH neuronal cells with 40 μM sphingomyelin for 24 h (n=6) and measured the expression of ABCA5 and alpha-synuclein by qRT-PCR and western blotting. The expression of both genes was significantly increased ($p<0.05$) with the sphingomyelin treatment. These new data provide further evidence indicating that lipid is important for alpha-synuclein pathology in PD.

POS-WED-103

SYNAPTIC DEGENERATION IN ALZHEIMER'S DISEASE

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Purpose: Synaptic dysfunction occurs early in Alzheimer's disease (AD) and is recognized as a primary pathologic target for AD treatment. Synapse degeneration or dysfunction contributes to clinical signs of dementia through altered neuronal communication and the degree of synaptic loss strongly correlates with cognitive impairment. The exact molecular mechanisms underlying synaptic degeneration are still relatively unclear; therefore, identifying abnormally expressed synaptic proteins in AD brain will help to elucidate those mechanisms and can lead to the identification of therapeutic targets that might slow AD progression. **Methods:** In the present study, synaptosomal fractions from post-mortem human brain tissue of AD (n = 6) and control (n = 6) were compared using 2D-differential in gel electrophoresis. AD pathology is region specific; human subjects can be highly variable in age, medication and other factors. Consequently, within each diseased state, two vulnerable areas (hippocampus and temporal cortex) were compared with two relatively spared areas (motor and occipital cortices). Proteins exhibiting significant changes in their expression were identified (≥20% change, *Newman-Keuls* P-value <0.05) using either MALDI-TOF or ESI-QTOF mass spectrometry. **Results:** Total of 28 different synaptic proteins exhibited greater than two-fold differences between expressions in AD and normal subjects. These proteins are involved regulating different cellular functions including energy metabolism, signal transduction, vesicle transport, structural and antioxidant function. **Conclusion:** This study showed that synaptic proteins in human AD brain are significantly different from those in control brain. Of the proteins that showed disease-specific expression changes, functions of septin-8, annexin A5, septin-11 and G(o) protein subunit-α involved in vesicle trafficking, transmembrane signaling and synaptic structure, respectively, with respect to AD will be discussed.

POS-WED-104

DIFFUSION AND T1 WEIGHTED IMAGING REVEALS CROSS-SECTIONAL AND LONGITUDINAL CHANGES IN NEUROPATHOLOGY OF HUNTINGTON'S DISEASE

Dominguez J.F.[1], **Egan G.F.**[1, 2, 3], Gray M.A.[1], Dymowski A.[1], Langmaid R.[1], Chua P.[1], Churchyard A.[4], Stout J.[1] and Georgious-Karistianis N.[1]
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Dominguez D, JF[1], Egan, GF[1, 2, 3], Gray, MA[1], Dymowski A[1], Langmaid, R[1], Chua, P[1], Churchyard, A[4], Stout J[1], Georgiou-Karistianis, N[1 1] School of Psychology and Psychiatry, Faculty of Medicine Nursing & Health Sciences, [2] Monash Biomedical Imaging (MBI), Monash University, Clayton, 3800; [3] Centre for Neuroscience, University of Melbourne, Parkville, 3052; [4] Department of Neurology, Monash Medical Centre, Clayton, 3168 **Purpose:** Huntington's disease (HD) is reflected in volume and diffusivity measures at the whole brain level and at the level of the basal ganglia and thalamus. This study aimed to identify T1 and diffusion based imaging biomarkers that track disease progression in pre-diagnosis and early manifest HD. **Methods:** T1 and diffusion weighted images were acquired twice, 18 months apart, in 36 controls, 35 pre-symptomatic HD (pre-HD) and 35 symptomatic HD (symp-HD) individuals. Volumetric, fractional anisotropy (FA) and mean diffusivity (MD) values in the whole brain, the basal ganglia and the thalamus were measured. **Results:** We found significant interactions between group and time in MD and FA measures ($p<0.05$). Caudate MD was higher in symp-HD vs pre-HD vs controls. It increased in symp-HD between baseline (Mean = 1.869 +/- 0.066 x 10^{-4} mm^2/s) and 18 months (2.014 +/- 0.079) but remained unchanged in controls and pre-HD. FA in the right putamen was higher in symp-HD vs. pre-HD vs. controls, and increased with time in pre-HD only. Group by time interactions were also observed in putamen and thalamus MD and thalamus FA. **Conclusions:** MD and FA can be sensitive biomarkers for detecting disease progression in HD even at the preclinical stage. Specifically, caudate MD and putamen FA can be used to discriminate between groups and additionally to track time-dependent changes in symp-HD and pre-HD respectively.

POS-WED-105

SEGMENTATION OF THE MOUSE STRIATUM AND PALLIDUM IN HIGH-RESOLUTION MAGNETIC RESONANCE IMAGES

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Purpose: The striatum and pallidum are subpallial nuclear groups that play an important role in motor, emotional and cognitive functions. They are often called the basal ganglia, but this antiquated term is elastic and unhelpful in the light of modern gene expression studies. To investigate the pathophysiology of disorders, high-resolution magnetic resonance (MR) imaging of mouse models is frequently employed. Although morphological changes in the mouse sub-pallium have been identified as disease biomarkers, a definitive delineation of the mouse striatum and pallidum is not available. In this study, we have created a detailed protocol for segmenting of the striatum and pallidum, in high-resolution MR images of the ex-vivo C57BL/6J mouse brain. **Methods:** Eighteen animals were perfused and fixed with 4% paraformaldehyde and 0.1% Magnevist®. Brains were extracted and incubated in 0.1% Magnevist/PB for 4 days, placed in and imaged on a 16.4T (89mm) Bruker micro-imaging system using a 15 mm SAW coil (M2M Imaging, USA). MRI data was acquired 3D gradient echo sequence with TR/TE/FA= 50ms/12ms/30°, 82 KHz spectral bandwidth, 8 excitations with an acquisition time of 5h 15mins to produce T1/T2*-weighted images at 30µm3 isotropic resolution. Subsequently, a symmetric model was created using a recursive non-linear hierarchical fitting strategy. **Results:** The components of the pallidum and striatum structures were delineated based on differences in signal intensity and/or their location in reference to other landmarks, and partitioned using vector-based segmentation. We were able to clearly identify the boundaries of the major striatal (caudate-putamen, accumbens, lateral striatal stripe, olfactory tubercle) and pallidal (globus pallidus, ventral pallidum) structures in the MR images. **Conclusion:** The result is a detailed guide for segmenting the mouse striatum and pallidum and a digital atlas containing over 20 structures with average region volumes and T2*-weighted signal intensities. (Other members of Australian Mouse Brain Mapping Consortium are Gary Egan, George Paxinos, Steve Petrou, and Perry Bartlett).

POS-WED-106

HIPPOCAMPAL VOLUME AND CELL DENSITY CHANGES IN A MOUSE MODEL OF GENETIC EPILEPSY

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Aim: A gene mutation in the $GABA_A$ gamma 2 receptor subunit ($GABA_A\gamma2_{R43Q}$) was discovered in patients with two major epilepsy phenotypes: childhood absence seizures and febrile seizures (FS). Using the $GABA_A\gamma2_{R43Q}$ mouse model we explored whether the R43Q mutation impacts beyond the moment-to-moment GABA mediated inhibitory events; including roles in regulating neuronal proliferation, migration and connectivity. We hypothesized that the R43Q mutation generates structural change, focusing on the hippocampus, a region vulnerable to epileptogenic features in animal models and in humans. $GABA_A\gamma2_{R43Q}$ mice in a C57Bl/6J background are a genetic model for FS susceptibility, without spontaneous seizures, providing an opportunity to measure the impact of genetic changes independent of on-going seizure activity. Methods: Thermal seizure susceptibility was assessed for R43Q and control mice. High-field MRI volume analysis of the hippocampus in adult brains was done using a 16.4T Bruker micro-imaging system. Histological analysis of MRI brains was used to obtain stereology-based estimates of neuron density for CA1-3 pyramidal cells and dentate granule cells (GrDG). Results: R43Q mice were significantly more susceptible to thermally triggered tonic-clonic seizures than control mice (n=12; p=0.0065). In R43Q animals, MRI determined volumes in GrDG and polymorph layers were approximately 5% greater (p= 0.04; p=0.03 respectively) and GrDG population estimates showed a 30% higher cell density (p=0.01) than controls (n=10 each). Conclusion: Our results suggest that R43Q mice have increased vulnerability to thermally-triggered seizures and that microstructural changes may contribute to hippocampal seizure susceptibility.

POS-WED-107

A HIGH CONTENT ASSAY TO MEASURE MICROGLIAL MORPHOLOGY

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Purpose: Microglia are critical cells in brain inflammation and many other physiological and pathological processes. A number of compounds, including macrophage colony-stimulating factor (M-CSF), alter microglial morphology and phenotype. Microglial morphology relates to function/phenotype but exactly how is presently unclear. We have developed an automated high content assay to quantify microglial morphology using the Metamorph image analysis program on images acquired using a Discovery-1 automated microscope. **Methods:** Adult human microglia, cultured from adult post-mortem brain and growing in 96-well microplates, were exposed to M-CSF (25 ng/ml) for 96 hours, fixed with 4% paraformaldehyde and then immunostained with an antibody to CD45 and Alexa-488. Images were acquired using a Discovery-1 automated microscope. A Journal (Microglial Shape) was written in Metamorph to measure microglial shape. The journal automatically thresholded each image to segment CD45-positive microglia, then applied the Integrated Morphometry Analysis tools Elliptical Form Factor (length/breadth) and Shape Factor ($4\pi A/p^2$, P = cell perimeter, A = cell area) to determine cell shape. These measures were then automatically logged to Excel spreadsheets. **Results:** Microscopic examination showed that M-CSF caused human microglia to assume a rod-like extended shape. To quantify this phenotypic change we applied the Microglial Shape Assay to images acquired from these microplates. M-CSF induced a statistically significant ($p < 0.01$) elongation of human microglia using both cell shape measures (Elliptical Form Factor and Shape Factor). **Conclusion:** We have developed an assay that can quantify Microglial shape at high throughput. This assay will be useful in studies relating microglial shape to function and for screening compounds that act on human microglial cells.

POS-WED-108

THE ANTIPSYCHOTIC-LIKE ACTION OF LSP1-2111, BUT NOT LY379268, IS DEPENDENT ON SEROTONERGIC SIGNALING, ACTING VIA THE 5-HT1A RECEPTORS

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Purpose: In the present experiments we investigated the involvement of serotonergic system in the mechanism of action of LY379268, group II metabotropic glutamate receptors (mGlu) agonist, and LSP1-2111, group III mGlu receptors agonist. We focused on the 5-HT1A receptors, which were shown to be involved in the antipsychotic-like activity. Methods: MK-801and amphetamine-induced hyperlocomotions, DOI-induced head twitches in mice, and the social interaction test in rats were used. LY379268 was given at the dose of 0.5 -1 mg/kg, 60 min before the test and LSP1-2111 was given at a dose of 0.3-5 mg/kg, 45 min before the test. 5-HT1A antagonist, WAY100635, was injected 45 min before the test, in a dose of 0.1 mg/kg. 8-OH-DPAT (0.01 mg/kg) was given 15 min before the test. All groups were n=10. Results: LY379268 showed clear antipsychotic-like action in all experiments. Pretreatment of the animals with WAY100635 had no effect on the activity of LY379268. In contrast, pretreatment with WAY100635 inhibited the action of LSP1-2111 ($p<0.01$ in MK-801-induced hyperlocomotion; $p<0.0001$ in amphetamine-induced hyperlocomotion; $p<0.009$ in DOI-induced head twitches and $p<0.05$ in the social interaction test). The co-administration of subeffective dose of LSP1-2111 (0.3 mg/kg) with subeffective dose of 8-OH-DPAT (0.01 mg/kg), resulted in clear antipsychotic activity reversing the deficits in all behavioural tests ($p<0.01$ in MK-801-induced hyperlocomotion; $p<0.009$ in amphetamine-induced hyperlocomotion; $p<0.0001$ in DOI-induced head twitches and $p<0.05$ in the social interaction test). Conclusions: The results indicate that the antipsychotic-like action of LSP1-2111, in contrast to LY379268, is dependent on the serotonergic system acting via 5-HT1A receptors.

POS-WED-109

ROLE OF ELECTRICAL SYNAPSES IN AMYGDALA DEPENDENT FEAR MEMORIES

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Purpose: The amygdala-hippocampal network plays a crucial role in fear learning and memory. The synchronized firing of some GABAergic interneuron populations, which are electrically coupled by gap junctions, tightly regulates activity within this network. We recently demonstrated that manipulations of electrical synapses (ES) in the dorsal hippocampus (DH) disrupted the formation of context-dependent fear memories and the emergence of CA1 theta rhythms *(Bissiere et al, 2011)*. While fear acquisition to a conditional stimulus tone was not affected by disruption of ES, tone fear extinction was accelerated when tested off blockers, suggesting that amygdala-depedent memories acquired or consolidated under ES blockade had been rendered more labile or prone to extinction. **Methods:** To test this hypothesis, we combined both systemic and intra-BLA infusions of gap junction blockers with auditory Pavlovian fear conditioning. The integrity of fear memories to a tone or a context was then tested. **Results:** Systemic blockade of ES using Carbenoxolone (Carb) in rats that were fear-conditioned using with a low footshock intensity uncovered a previous ceiling effect and resulted in rapidly fading tone fear acquisition (Carb n=9; Veh n=9). These effects were further confirmed by manipulating gap junctions in the basolateral amygdala, which impaired the acquisition (n=12 all groups), consolidation (n=6 all groups) and the expression of tone fear memories (n=6 all groups) as well as the acquisition and consolidation of context fear memories (n=8 all groups). **Conclusion:** Thus, we propose a role for electrical synapses on amygdala-dependent fear behaviors, and also more generally on mechanisms regulating the fear circuitry and processes of learning and memory.

POS-WED-110

LONG-TERM EFFECTS OF TAFAMIDIS FOR THE TREATMENT OF TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY

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Background and Aims: Tafamidis, a small molecule that kinetically stabilises transthyretin (TTR), slowed disease progression in patients with TTR familial amyloid polyneuropathy (TTR-FAP) in an 18-month, randomised, double-blind, placebo-controlled study. The objectives of the present open-label extension study were to assess the long-term efficacy, safety, and tolerability of tafamidis and to evaluate whether earlier initiation yields clinical benefits. **Methods:** Patients with V30M TTR-FAP (n=86) who had received tafamidis or placebo for 18 months were given tafamidis 20 mg/day for 12 months. Efficacy measures included the Neuropathy Impairment Score - Lower Limbs (NIS-LL), Norfolk total quality of life (TQOL) score, and large- and small-nerve fibre function. The monthly rates of change in each end point were compared between the original and extension studies, and treatment-related adverse events (AEs) were monitored. **Results:** Patients who switched from 18 months of placebo to 12 months of tafamidis experienced a slowing of the monthly rate of change in NIS-LL (from 0.34/month to 0.16/month; P=0.0103), TQOL (from 0.61 to -0.16; P=0.0003), and large- (from 0.18 to 0.11; P=0.2133) and small-nerve fibre function (from 0.09 to 0.04; P=0.0551). Patients who continued on tafamidis experienced stable rates of change in NIS-LL (from 0.08/month to 0.11/month; P=0.6000), TQOL (from -0.03 to 0.25; P=0.1632), and large- (from 0.06 to 0.05; P=0.9298) and small-nerve fibre function (from 0.03 to 0.05; P=0.3348). Patients administered tafamidis for 30 months had 55.9% greater preservation of function, as determined by the NIS-LL, and large- and small-nerve fibre function (66.0% and 45.5%, respectively) than patients who began tafamidis 18 months later. The most common treatment-related AE was headache (4.7%). **Conclusions:** The treatment effect of tafamidis was sustained over 30 months, tafamidis was safe and well tolerated, and earlier initiation preserved neurologic function. This study was sponsored by FoldRx Pharmaceuticals, acquired by Pfizer Inc in October 2010.

POS-WED-111

CAN STRENGTH MODELS EXPLAIN SELF-REGULATION IN COGNITION?

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Purpose: Recently, there has been a surge of research suggesting that self-regulation, across various domains ranging from cognition to social processes, draws upon a common depletable resource. This research gives, credence to one of the most contentious models of recent times: the strength model of self-regulation. These studies aimed to further explore the interplay between cognition and self-regulation depletion, and identify the cognitive factors that influence self-regulation resources. **Method:** Study 1 (n=35) employed a repeated measures dual task paradigm to assess changes in self-regulation across unrelated cognitive tasks. All participants completed an arithmetic task and an analogous sign discrimination task (as a control task) in a counterbalanced order. Both the arithmetic and the control task were followed by the same stop-signal task (SST) to assess motor response inhibition. In study 2 (n=22), a simple detection task was used as a control for the arithmetic task. **Results:** Evidence of stop-signal reaction time (SSRT) depletion was observed for individuals with low accuracy measures on the arithmetic task, which quickly diminished across the block of the SST, $F(2,66)=4.13$, $p=.02$. Interestingly, motor responses (part $r=-.43$, $p=.002$) and switching costs between arithmetic operations (part $r=.48$, $p=.001$) on the simple discrimination (but not the detection) task could also predict SSRD depletion independent of arithmetic ability. **Conclusion:** The depletion of self-regulation across cognitive tasks is short lived, dynamic and variable with cognitive ability. These findings also suggest that simple discrimination tasks can deplete and mask changes associated with self-regulation in dual-task paradigms.

POS-WED-112

QUANTIFICATION OF VESICULAR GLUTAMATE AND GABA TRANSPORTERS IN ALZHEIMER'S DISEASE BRAIN

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Purpose: Synaptic loss and changes in synaptic proteins correlate closely with disease progression and severity in Alzheimer's disease (AD). Glutamatergic neurons are particularly vulnerable. Recent studies suggest that the vesicular glutamate transporter proteins are affected early in AD pathogenesis. This study quantified the mRNA expression of the vesicular glutamate transporter VGLUT1 and the vesicular inhibitory amino acid transporter VIAAT, as well as the synaptic terminal marker synaptophysin, in human autopsy brain tissue from AD and control cases. **Methods:** VGLUT1, VIAAT and synaptophysin were quantified in human autopsy brain tissue from AD cases (n=12) and neurologically normal controls (n=11) using real-time RT-PCR. Three regions were examined, the hippocampus, inferior temporal cortex (ITC) and occipital cortex. The hippocampus and ITC are severely affected by AD pathology, whereas the occipital cortex is relatively spared. **Results:** VGLUT1, VIAAT and synaptophysin transcripts were not significantly different between AD cases and controls in any of the regions studied. The comparable expression of the synaptic marker synaptophysin between groups, even in regions severely affected by AD such as hippocampus and ITC, indicates that there is either incomplete Wallerian degeneration or a florid resprouting of terminals. Our data suggest that in these terminals there is no change in expression of either vesicular transporter transcript. **Conclusion:** We found no change in the transcript expression of VGLUT1, VIAAT or synaptophysin in AD cases when compared with controls. If the protein levels follow the mRNA data, this would indicate that functional presynaptic terminals could be present within damaged regions of the AD brain.

POS-WED-113

VACCINATION AGAINST TAU SLOWS DISEASE PROGRESSION IN AGED P301L TAU TRANSGENIC MICE

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Purpose: In Alzheimer's disease (AD) brains, the microtubule-associated protein tau deposit as intracellular neurofibrillary tangles (NFTs). Tau deposits are furthermore found in a significant number of frontotemporal dementia (FTD) cases. Unfortunately, current therapeutic options a very limited. Active immunization against pathogenic tau has only recently been shown to prevent pathology in young tau transgenic mice, when treated before the onset of disease. However, in humans, diagnosis and treatment would be routinely done when symptoms are overt, meaning that the histopathological changes have already progressed. **Methods:** We used active immunization to target pathogenic tau in 4-, 8-, and 18 months-old P301L tau transgenic mice, a model of AD and FTD, that have an onset of NFT pathology at 6 months of age. After up to 10 months of treatment, brains were analysed histologically to determine the degree of NFT pathology. **Results:** In all age groups, NFT pathology was significantly reduced in treated compared to non-immunized mice. Similarly, phosphorylation of tau at pathological sites was reduced. In addition, increased astrocytosis was found in the oldest treated group. **Conclusion:** Our data suggests that tau-targeted immunization slows the progression of NFT pathology in a mouse model of tau pathology.

POS-WED-114

STUDY OF THE DISRUPTION OF NERVE-CELL CONNECTIONS IN ALZHEIMERS DISEASE THROUGH THE ASSAY OF TRANS-SYNAPTIC PROTEIN NEUROLIGIN2

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Background: Synaptic damage is one of the most important hallmarks of Alzheimer's disease (AD), and is the best correlate of cognitive impairment. Synapses are a key site of regulation between neurons and are characterized by different protein complexes arranged at tightly apposed pre- and postsynaptic terminals. The best-established trans-synaptic complex involved in synaptogenesis comprises the binding between presynaptic neurexins (NRXNs) and postsynaptic neuroligins (NLGNs). Fluctuations in the levels of these protein would sway the balance between excitatory or inhibitory neurotransmission in the brain. An imbalance favouring over-excitation, either through an over-abundance of excitatory, or an under-representation of inhibitory synapses could lead to damage of synaptic and dendritic damage, and ultimately to neuronal death via glutamate-mediated excitotoxicity.Aims: Investigate the disruption of nerve-cell connections in Alzheimer's disease through the assay of the trans-synaptic protein Neuroligin 2 and correlate it to the pathological severity of the disease. Methodology: Neuroligin-2 protein was quantified in 3 brain areas that differ in susceptibility to neuronal loss in AD, in autopsy tissue from 15 control subjects and 15 patients with pathologically confirmed AD. Quantification was conducted by in-gel immunodetection against known concentrations of recombinant truncated neuroligin-2 standards. Results: Plot data showed that Neuroligin2 quantity was significantly lower in AD cases than in controls. Area based analysis showed that the level of Neuroligin2 in occipital cortex was significantly lower in female AD cases (222.57 ng/mg of total protein) than in sex and age matched controls (401.7 ng/mg of total protein). Correspondingly, Neurolign2 level in inferior temporal cortex was reduced in AD cases (123.4 ng/mg of total protein) than in aged and sex matched controls (123.4 ng/mg of total protein). In addition, novel splice variants of Neuroligin2 were identified in all selected brain areas. This is the first study of Neuroligin2 expression in AD. Conclusion: This study indicates a potential role for neuroligin2 in AD pathogenesis. The reduction in neuroligin2 quantity could implicate in inhibitory synapses dysfunction, which could guide to, excitotoxic damage in AD.