POS-TUE-001

ADULT CANINE SKIN-DERIVED AND BRAIN-DERIVED NEUROPRECURSORS: AN IN VITRO COMPARISON

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PURPOSE Neuroprecursors have been isolated from the skin and brain of several mammalian species, including the domestic dog *(Canis familiaris)*. (Valenzuela et al, 2009). Skin and the major cell types of the brain all arise from the embryonic ectoderm. Apart from this shared lineage, the similarities between skin-derived and brain-derived neuroprecursors remain unclear. The aim of the study was to compare the proliferation capacity and differentiation potential of skin-derived and brain-derived neuroprecursors under in vitro culture conditions. **METHODS** Periventricular regions were dissected from fresh *post mortem* canine brain and neuroprecursors isolated according to (Bull et al, 2006). Skin derived precursors were isolated and propagated according to our protocol (Valenzuela et al 2009). Proliferative potential was quantified using 5-ethynyl-2'-deoxyuridine (EDU, Invitrogen). Spontaneous neuronal differentiation was induced by removal of the mitogens and addition of 10ng /mL of Brain Derived Neurotrophic Factor (BDNF) to culture medium. Gene and protein expression for neuronal markers were carried out using PCR and immunocytochemical staining respectively. **RESULTS** Canine skin and brain-derived neuroprecursors are morphologically similar under proliferation conditions and express neural stem cell markers *Nestin, NCAM and CD133*. EDU assays revealed significant decreases ($p<0.05$) in the proliferation rates of skin-derived (32.63%) compared to brain-derived (74.64%) neuroprecursors after the third passage. Spontaneously differentiated cultures from both sources expressed mature neuronal markers *βIII tubulin*, *MAP2* and *NSE*. However, a higher density of glial marker *GFAP* positive cells in brain-derived cultures (>90%) than skin-derived (<1%) was observed (N=4). Additionally, expression of GABAergic marker GAD67 was limited to skin-derived samples. Immunocytochemical findings were corroborated by PCR analysis. **CONCLUSIONS** Several similarities exist between canine skin and brain-derived neuroprecursors in terms of morphology and expression of neural stem cell markers. Skin-derived neuroprecursors exhibit significantly decreased long term proliferation rates and have a decreased capacity for glial differentiation *in vitro*.

POS-TUE-002

$GABA_A$ RECEPTOR α-SUBUNIT EXPRESSION ACROSS DEVELOPMENT IN THE PIGLET BRAIN

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Purpose: GABA is one of the major neurotransmitters in the mammalian brain. The principal function of the GABA system in mature brain is inhibition, however in the neonatal brain GABA provides much of the excitatory drive. The switch in function occurs early in the postnatal period in rats; however it is unknown precisely when this switch occurs in the neonate. The piglet is ideal for investigating human brain development; with many of the neurodevelopmental processes such as synaptogenesis, neuronal migration and myelination occurring at a similar schedule to that in humans. We aimed to assess changes in protein expression of the $GABA_A$ receptor α_1 α_2 and α_3 subunits in the piglet brain in the perinatal period. **Methods**: $GABA_A$ receptor α_1, α_2, and α_3 protein expression levels were analysed by western blot. Preterm piglet brain tissue was obtained through caesarean section, P0 and P7 tissue was obtained from piglets delivered naturally (n=5). Brains were removed, sectioned and frozen in 0.32M sucrose for western blot analysis. **Results**: $GABA_A$ α_1, α_2, and α_3 protein expression changed across development, with a switch in the dominant α-subunit observed in four cortical regions (frontal, parietal, temporal and occipital). Birth appeared to have a strong effect on subunit expression, with significant increases in α_1 and α_3 expression at P0 when compared with expression at P-1. **Conclusions**: Knowledge of which $GABA_A$ receptor subunits α-isoforms are abundant in the developing brain is critical to understanding and developing effective seizure treatment strategies specific to the neonatal brain.

POS-TUE-003

CORTICAL INTERNEURON POSITIONING AND LAMINATION ARE ALTERED IN BARREL FIELD MUTANTS

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Purpose: The maturation of inhibitory circuits is dependent on sensory experience and interneurons migrate and reside in specific laminar locations corresponding to birthdate. Interneurons destined for cortical layer IV are generated at embryonic day (E) 14.5 and our previous studies have shown that these neurons reach a final position in the second postnatal week, a time corresponding with formation of the barrel field. It is unclear whether changes in pyramidal neuron cytoarchitecture or synaptic activation by thalamocortical axons are the driving stimulus for recruitment. **Methods:** Interneuron subtype specification and laminar positioning were characterized in two barrel field mutants, adenylyl cyclase 1 (AC1) and phospholipase C-beta1 (PLC-beta1) knockouts. To determine the timing of mid-born interneuron final positioning, a BrdU-birthdating study was conducted. **Results:** Characterization of the AC1 mutants during the first postnatal week revealed no changes within layer IV, but an increase in the proportion of calbindin (CB)-positive cells in layer V when compared to the wildtype littermate control (n = 3 in each group). The number of CB-positive cells was unaltered in layer VI, however, these cells exhibited an abnormal distribution and were arranged in clusters of 4-5 neurons instead of the even distribution in the wildtype cortex. The birthdating study on the PLC-beta1 mutants revealed alterations in the timing of final positioning at P7 with earlier interneuron recruitment compared to the wildtype. **Conclusion:** This study provides new insight on how barrel field development is linked to the recruitment of interneurons into specific cortical layers and emphasizes the role of sensory experience in the formation of cortical circuits.

POS-TUE-004

BACURD2 IS A NOVEL INTERACTING PARTNER TO RND2 WHICH REGULATES THE CELL MORPHOLOGY AND MIGRATION OF EMBRYONIC CORTICAL NEURONS

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Rnd2, a member of the atypical Rho-like GTP-binding family of proteins, is critical in the initiation of neurite outgrowth as well as cell migration by newborn neurons of the embryonic cerebral cortex by suppressing RhoA protein signaling (Heng et al, Nature, 2008, Pacary et al, Neuron, 2011). However, the underlying molecular mechanisms remain poorly understood. **Purpose**: To characterise the signaling pathways of Rnd2 through identifying its protein interacting partners during mouse cortical development. **Methods**: Yeast two-hybrid assays were employed to search for Rnd2 interacting partners, which were then confirmed by co-immunoprecipitation. The expression in the developing mouse brain was verified using in-situ hybridisation and in utero electroporation at embryonic day 14 was performed to study the functions of these putative binding partners. **Results**: Our investigations identified Bacurd2 as a binding partner to Rnd2 and their interaction was confirmed in transiently transfected HEK293T cells. Both proteins were expressed in developing mouse brain and in utero electroporation demonstrated that changes to Bacurd2 expression levels significantly affect the migration of neurons (>5000 cells counted per condition and n=3-4 per treatment). Deletion mapping studies revealed Bacurd2 including its Rnd2 binding domains to be important for the regulation of actin cytoskeleton in HeLa and primary neuronal culture when labelled with phalloidin and utrophin respectively. Further in utero electroporation studies confirmed these sub-domains to be important for the migration of neurons in embryonic cortex as well as regulating the morphology and neurites lengths of PC12 cells. **Conclusion**: We identified Bacurd2 to be a novel interacting partner of Rnd2 and is important for the regulation of neurite outgrowth and cell migration. We suggest that Bacurd2 associates with its binding partners, including Rnd2, to regulate the actin cytoskeleton and cell morphology of neurons.

POS-TUE-005

ENVIRONMENTAL ENRICHMENT INFLUENCES MATURATION OF PARVALBUMIN-POSITIVE INHIBITORY INTERNEURONS WITHIN THE STRIATUM

O'Connor A.M., Leamey C.A. and Sawatari A.
Bosch Institute, University of Sydney, NSW, 2006.

Purpose: The critical period is the time of peak plasticity within the nervous system. Parvalbumin positive (PV+) inhibitory interneurons are thought be vital in regulating the timing of this important developmental epoch, consolidating circuits formed by maturing excitatory neurons. In turn, PV+ inhibitory interneurons may themselves be influenced by cellular correlates of inhibitory interneuron maturation, such as brain-derived neurotrophic factor (BDNF). This study investigates whether environmental enrichment influences morphological development of PV+ inhibitory interneurons and BDNF protein levels 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 were measured for PV+ cells within the striatum. The striatum was dissected from fresh brains at the same ages (n=4), and homogenised. Levels of BDNF protein were determined using an enzyme-linked immunosorbent assay (ELISA). A univariate ANOVA was used to compare values, with environment and age as between-subjects factors. **Results:** As animals matured, there was a significant interaction between environmental condition and age on soma size of PV+ inhibitory interneurons ($p=0.005$). At P10, EE animals showed a significantly larger mean soma size than SE animals ($p=0.015$). At a young age (P10 – P15), EE animals displayed a greater amount of BDNF within the striatum than SE animals ($p<0.05$). **Conclusion:** These results suggest that maturation of PV+ inhibitory interneuron circuitry within the striatum is affected by age and environment. Environmental enrichment may regulate timing of the critical period by accelerating the maturation rate of these neurons, whilst also influencing cellular correlates of this maturation.

POS-TUE-006

THE ROLE OF NEOGENIN IN THE GENERATION OF ADULT BORN NEURONS IN THE OLFACTORY SYSTEM

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Purpose: In the adult rodent brain neuroblasts migrate along the rostral migratory stream towards the olfactory bulb and integrate into granule cell and glomerular layers of the olfactory bulb. The trans-membrane receptor Neogenin is a member of the immunoglobulin superfamily and is expressed by progenitor cells in the adult mouse brain. Neogenin loss of function ($neo^{gt/gt}$) mice exhibit smaller olfactory bulbs. Quantification of neuronal subsets revealed that the number of calretinin-positive cells in the olfactory bulb were significantly lower in the neogt/gt when compared to $neo^{+/+}$. However, there was no change in the calbindin or tyrosine hydroxylase positive interneuron populations. These observations lead to the hypothesis that neogenin plays a role in the generation of adult born interneurons. **Methods:** To test this hypothesis a quantitative analysis was carried out on the olfactory bulbs of $neo^{gt/gt}$ and $neo^{+/+}$ mice injected with bromodeoxyuridine (brdU). Mice were analysed 2 hours and 7 days later. BrdU positive cells were co-labelled with Pax6, doublecortin (DCX) and Neun, which are expressed by progenitors, migrating neuroblasts and mature neurons respectively. Co-expression of these markers with BrdU was quantified along the rostrocaudal axis (SVZ – RMS – OB). **Results:** This analysis revealed that after a 2 hour BrdU pulse there was a significantly higher number of $Pax6^+/BrdU^+$ ($p=<0.05$) and $DCX^+/BrdU^+$ ($p<0.01$) proliferating progenitors in the rostral migratory stream of the $neo^{gt/gt}$ ($n=3$). **Conclusion:** Together these data suggest a role for neogenin in migration along the RMS or that neogenin may regulate neuronal proliferation and differentiation. Further analysis will be carried out to investigate the precise mechanism by which neogenin regulates adult neurogenesis.

POS-TUE-007

THE MOLECULAR ROLE OF GTF2IRD1 IN THE WILLIAMS-BEUREN SYNDROME COGNITIVE PROFILE

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Williams-Beuren syndrome (WBS) results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. Its features typically involve 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 discovered by us, encoded by the *GTF2IRD1* gene, as a principal player in the origin of these neurological defects. **Purpose:** To explore the basis of GTF2IRD1's role, we generated *Gtf2ird1* knockout mouse lines that show some developmental defects that are strikingly similar to WBS and we are interrogating the molecular and cellular mechanisms that underpin these phenotypic abnormalities. **Methods:** Knockout analysis has involved a battery of behavioural testing, microarray screening and detailed expression mapping. Analysis of GTF2IRD1 function includes DNA binding assays and protein-protein interaction studies. **Results:** Behavioural analysis showed evidence of defects in motor coordination, hyperactivity, social engagement and context-specific anxiety and we have mapped *Gtf2ird1* expression to brain regions that support these phenotypes, including cerebellum, basal ganglia and limbic system. Our microarray analysis (5KO v 5WT) has revealed enhanced activation of a set of immediate early genes, potentially correlating with the observed hyperactivity. DNA binding and protein-protein interaction studies have shown auto-regulatory control of gene transcription and interactions with chromatin modifying proteins. **Conclusion:** Our work positions GTF2IRD1 as a new epigenetic regulator of neuronal differentiation and function with important consequences for the understanding of human behaviour.

POS-TUE-008

CHARACTERIZATION OF SPIRAL GANGLION NEURITE DEGENERATION AND REGENERATION IN SITU USING HIGH FIELD MRI

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Purpose: High field MRI provides the opportunity to evaluate neural remodelling of the cochlea associated with sensorineural hearing loss while preserving structural integrity. We undertook an evaluation of the changes in spiral ganglion afferent neurites arising from lesion of the sensory hair cells in the organ of Corti through combination ototoxic treatment. **Methods:** Ototoxic treatment under isoflurane anaesthesia produced selective loss of the cochlear sensory hair cells and subsequent atrophy of the peripheral neurites of the spiral ganglion primary afferents. After two weeks, the left cochleae were treated with the neurotrophin BDNF via transduction of mescenchymal cells with a BDNF gene construct to promote regeneration of the neurons. A further two weeks later the animals were euthanized with pentobarbital and left (BDNF treated n = 2) and right (untreated n = 2) cochleae were collected and fixed in paraformaldehyde and then placed in 0.2% Magnevist in normal saline. 3D structural gradient echo images were acquired using a Bruker AV700 16.4T MRI system using a 5 mm volume coil in a micro5 gradient set. 3D gradient echo, FLASH, images were acquired with the following parameters: TR = 40, TE = 5.5, pulse = 35°, spatial resolution = 12.5 X 12.9 X 12.5 µm, ns = 4. Additional parallel experiments using conventional confocal laser scanning microscopy (Zeiss 710 NLO LSM) for β-tubulin immunofluorescence in 50 µm cryosections (n = 4) validated MRI imaging. **Results:** The MRI image stacks enabled complete digital resection of the cochlea which yielded high resolution longitudinal slices that clearly resolved the cochlear partition, including the Reissner's membrane bilayer separating scala media from scala vestibuli. In the region of Rosenthal's canal and the osseous spiral lamina, there was a clear difference in the integrity of the spiral ganglion radial fibre projection of nerve fascicles. The untreated cochleae showed a reduction in the neural fibre density in the osseous spiral lamina, whereas the BDNF-treated cochleae showed a strong fibre track signal. This regenerated fibre track extended beyond the habenula perforata towards the lateral wall (spiral ligament), with evidence for fibre branching and extension into the underlying perilymphatic compartment (scala tympani). **Conclusion:** The high field MRI enabled determination of the full extent of the regenerated nerve fibre field within the reconstructed cochlea.

POS-TUE-009

THE RNA-BINDING FACTOR ZFP36L1 IS A DOWNSTREAM TARGET OF NFIX DURING CORTICAL DEVELOPMENT

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Neural progenitor cells have the ability to give rise to neurons and glia in the embryonic, postnatal and adult brain. During development the program regulating whether these cells divide and self-renew or exit the cell cycle and differentiate is tightly controlled, and imbalances to the normal trajectory of this process can lead to severe functional consequences. However, our understanding of the molecular regulation of these fundamental events remains limited. Our recent work has revealed that the transcription factors of the Nuclear Factor One (Nfi) family play a central role in promoting neural progenitor cell differentiation during cortical development, however, the factors downstream of the Nfi family that mediate this process remain unclear. Here we show that the RNA binding protein, Zfp36L1, is a target for transcriptional regulation by Nfix during cortical development. Zfp36L1 is strongly and specifically expressed by cortical neural progenitor cells, and its expression is upregulated in the cortex and hippocampus of Nfix knockout mice at both the mRNA and protein level. Moreover, in silico analysis of the Zfp36l1 promoter reveal the presence of an NFI binding site within the proximal promoter region of this gene. Collectively, these findings suggest that Nfix may promote neural progenitor cell differentiation, in part, via the transcriptional regulation of Zfp36l1.

POS-TUE-010

EFFECTS OF EXOGENOUS IGF2 ON NEURAL DIFFERENTIATION OF PARTHENOGENETIC MOUSE EMBRYONIC STEM CELLS

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Purpose: Differential capacity of the parthenogenetic embryonic stem cells (PESCs) is still under controversy and the mechanisms of its neural induction are yet poorly understood. Here we demonstrated neural lineage induction of PESCs by addition of insulin-like growth factor-2 (Igf2), which is an important factor for embryo organ development and a paternally expressed imprinting gene. **Methods:** Murine PESCs were aggregated to embryoid bodies (EBs) by suspension culture under the leukemia inhibitory factor-free condition for 4 days. To test the effect of exogenous Igf2, 30 ng/ml of Igf2 was supplemented to EBs induction medium. Then neural induction was carried out with serum-free medium containing insulin, transferrin, selenium, and fibronectin complex (ITSFn) for 12 days. Normal murine embryonic stem cells derived from fertilized embryos (ESCs) were used as the control group. Neural potential of differentiated PESCs and ESCs were analyzed by immunofluorescent labeling and real-time PCR assay (Nestin, neural progenitor marker; Tuj1, neuronal cell marker; GFAP, glial cell marker). **Results:** The differentiated cells from both ESC and PESC showed heterogeneous population of Nestin, Tuj1, and GFAP positive cells. In terms of the level of gene expression, PESC showed 4 times higher level of *GFAP* expression than ESCs ($p<0.05$). After exposure to Igf2, the expression level of *GFAP* decreased both in derivatives of PESCs and ESCs. Interestingly, the expression level of *Tuj1* increased only in ESCs, not in PESCs ($p<0.05$). **Conclusion:** The results show that IGF2 is a positive effector for suppressing over-expressed glial differentiation during neural induction of PESCs and for promoting neuronal differentiation of ESCs, while exogenous Igf2 could not accelerate the neuronal differentiation of PESCs. Although exogenous Igf2 promotes neuronal differentiation of normal ESCs, expression of endogenous *Igf2* may be critical for initiating neuronal differentiation of pluripotent stem cells. Supported by NRF (2012-0006145), Korea.

POS-TUE-011

TOWARD A CELL-REPLACEMENT THERAPY FOR HIRSCHSPRUNG DISEASE

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Hirschsprung Disease (HSCR) is characterised by an absence of enteric neural crest (NC) derived ganglia (composed of neurons and glia) in the distal portion of the bowel. This results in a failure of effective peristalsis in the aganglionic gut segment. Currently, HSCR is treated by surgical resection of the aganglionic bowel segment and adjacent ganglionated region. However, it is possible that introduction of endogenous NC stem/progenitor cells into the aganglionic bowel could restore peristaltic function. Towards this aim we have developed an effective method for isolation of NC cells from resected patient bowel tissue. In this method colon tissue cells are initially cultured as a monolayer in a defined neural media. The target NC cells can then be isolated by live-cell labeling and FACS with antibodies raised against either HNK1 or p75. When interrogated by immunocytochemistry and qPCR these NC-sorted cells are strongly enriched in NC markers which include SOX10, HuCD neuronal protein, and S100beta glial protein. Human NC cells can be transduced with a lentivirus-GFP reporter and when engrafted to patient-matched endogenous HSCR aganglionated muscle deposit both neural and glial components (N=4). Current tests will establish if the transplanted neural cells are capable of producing action potentials or initiating more coordinated peristaltic contractions in the aganglionated colon tissue. In addition, we are investigating the use of a degradable polymer scaffold to allow efficient clinical delivery of NC cells into HSCR colon. In pilot animal studies we have shown we can re-introduce NC cells into the colonic wall of an aganglionated colon when the colon is wrapped with a polymer scaffold seeded with NC cells.

POS-TUE-012

RECOVERY OF VISUALLY-DRIVEN ACTIVITY IN ENVIRONMENTALLY ENRICHED TEN_M3 KNOCKOUT MICE

Savvas L., Sawatari A. and Leamey C.A.
Physiology, School of Medical Sciences and Bosch Institute, University of Sydney, NSW, 2006.

Purpose: Ten_m3 deletion causes a mismapping of ipsilateral retinogeniculate projections, leading to suppression of activity in the primary visual cortex (V1). This study aimed to determine whether plasticity induced by environmental enrichment can promote a restoration of visually-driven activity in V1. Methods: Wild-type (WT) or Ten-m3 knock-out (KO), aged 3-6 months or 17-22 months were enriched or standard housed for 6-8 weeks. On the day before sacrifice, animals were placed in darkness and transferred to a light environment for 2 hours prior to euthanasia and perfusion. C-fos immunohistochemistry was performed and the percentage area of positive label within layer IV of V1 calculated. Anterograde tracing of retinal projections with choleratoxin subunit B was performed in some animals enriched from birth. Results: In contrast to our previous findings showing that c-fos levels are markedly depleted in Ten_m3 KOs compared to WTs[1], we found c-fos levels were similar between enriched young adult KOs (n=3) and WTs (n=4) (KO: mean=0.347, SD=0.103; WT: Mean=0.368, SD=0.130, p=0.678), suggesting a recovery of visually-driven activity. In aged mice, c-fos levels were significantly lower in enriched KOs (n=4) compared to WTs (n=4) (KO: Mean=0.223, SD=0.134; WT: Mean=0.302, SD=0.108, p=0.009). Expression was qualitatively greater than in non-enriched KOs, suggesting a partial recovery. Interestingly, staining appeared patchy in aged enriched KOs suggesting that one eye had become dominant. Preliminary retinogeniculate tracing data suggests that some aspects of the recovery may occur subcortically in young mice. Conclusion: Environmental enrichment leads to recovery of visually-driven c-fos activity in young Ten_m3 KO adults. Some capacity for recovery may also be present in aged mice. 1. Merlin et al., 2012, Cerebral Cortex, *Bhs030*.

POS-TUE-013

PROLIFERATIVE ENRICHMENT OF HUMAN SKIN-DERIVED NEURAL PRECURSOR CELLS USING BETACELLULIN

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Purpose: Neural precursor cells obtained from the stem cells of adult skin provide a good source of multipotent and progenitor type cells for therapeutic cell-based therapy. However, the isolation of these specialized cells in humans is complicated by low efficiency. Here we show that Betacellulin, an EGF derivative, promotes the proliferation of Nestin+ cells from adult human skin. **Methods:** A heterogenous dermis-derived progenitor cell population was isolated and allowed to form free-floating neurospheres in culture. Specialized media conditions DMEM/F12 supplemented with bFGF, EGF, B27, Glutamax, Sodium Pyruvate, Sodium Bicarbonate and Heparin with the addition of Betacellulin. Cells were seeded on collagen coated plates to allow for optimal conditions. EDU assays to measure the level of proliferation, along with immunocytochemistry and QPCR for gene expression were carried out at n=3. **Results:** The addition of betacellulin significantly increased the number of neurospheres, increased fraction of EDU+ cells, as well as up regulated gene expression of neural stem cell markers such as Nestin and HES1. **Conclusion:** Taken altogether, the addition of betacellulin to culture conditions stimulates the quiescent stem and progenitor cells and leads to overall greater neural precursor proliferation. This method may be helpful for improved culture of neural precursor cells from human adult skin.

POS-TUE-014

THE EFFECT OF MENINGEAL CELLS ON DOPAMINERGIC FETAL TISSUE GRAFTS IN PARKINSONIAN MICE

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Background: Dopaminergic neuroblasts, isolated from ventral midbrain (VM) fetal tissue, have been shown to functionally integrate and alleviate Parkinsonian symptoms in animal and clinical trials. Recently we, and others, have demonstrated that the use of donor tissue isolated at an age younger than conventionally employed can result in significantly more dopaminergic neurons within the graft – a consequence of improved cell survival and neuroblasts proliferation at the time of implantation. However within these studies little attention was made to remove the overlying meninges from the younger tissue, due to its 'sticky' attachment to the brain at this early stage in development. Of relevance, studies have shown that the meninges are far more than strictly a protective layer and that these cells serve as signaling centers, secreting a variety of factors that instruct surrounding tissues in the course of developmental programs. Consequently it remains to be determined what impact meningeal cells have on grafted dopaminergic neuroblasts. **Methods:** We examined the effects of culturing young (embryonic day, E10) verses older (E12) VM tissue in the presence or absence of E10 or E12 meningeal cells. Additionally, Parkinsoniam mice received grafts of E10 or E12 VM tissue +/- meninges. **Findings:** In culture we show that young, but not older VM tissue is responsive to the presence of meningeal cells, resulting in increased numbers of dopaminergic neurons and neurite length. Upon transplantation, E10, but not E12, donor tissue was responsive to the presenc of meninges – resulting in larger grafts and greater neurite outgrowth. On going studies are now required to identify the signalling molecules (and/or scaffolding) responsible for these effects.

POS-TUE-015

EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID MODULATE NEUROGENESIS IN A HUMAN HIPPOCAMPAL PROGENITOR CELL LINE AND PREVENT CORTISOL INDUCED STRESS-RESPONSE

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Purpose: It is now well established that during adulthood, new neurons are generated from adult neural stem cells residing in the dentate gyrus of the hippocampus, a region important for memory and learning function as well as mood in rodents and humans. In the rodent, an increase of neurogenesis in the hippocampus is associated with improved memory and learning abilities, whereas increased levels of Cortisol and a decreased neurogenesis are associated with symptoms of depression. The level of Adult Hippocampal Neurogenesis (AHN) can be regulated by factors such as enriched environment, physical activity, aging, and stress but also by diet. The Omega-3 fatty acids Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are known to have beneficial effects on cognition and mood; these effects are hypothesised to be mediated via modulating AHN. **Methods:** We explored the molecular mechanisms by which EPA and DHA modulate AHN using a human hippocampal progenitor cell line (HPC) (n=3) in an *in vitro* model of stress. Cells were pretreated with EPA (10μM) or DHA (10μM) for 3 days and then treatment with Cortisol at high, stress relevant (100μM) concentrations was started. **Results:** We show that, EPA and DHA pre-treatment prevents the detrimental effects of Cortisol on proliferation and neurogenesis by significantly increasing the percentage of dividing cells and neurogenesis while significantly decreasing apoptosis mainly by promoting survival. **Conclusion:** This study provides for the first time evidence in a human *in vitro* model of neurogenesis and stress that EPA and DHA can modulate neurogenesis and prevent its decrease induced by stress.

POS-TUE-016

COMPLEX COMPLEMENTARY ROLES OF TEN-M2 AND TEN-M4 IN REGULATING IPSILATERAL RETINAL PROJECTIONS

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Purpose: Teneurins (Ten-ms) are transmembrane glycoproteins which regulate the formation of neural circuits. Previous work has shown opposing roles for Ten-m2 and Ten-m4 in regulating binocular circuits, with Ten-m2 knockout (KO) mice showing a decreased ipsilateral projection from ventral retina correlating with reduced projections to rostral dorsal lateral geniculate nucleus (dLGN). Ten-m4 KOs show an increased projection from temporal retina with expanded projections to caudal dLGN. Here we investigate Ten-m2 and Ten-m4 heterozygotes and Ten-m2/Ten-m4 double KOs to determine potential interactions between these two genes. **Methods:** Choleratoxin B conjugated with a green or red fluorophore was injected into the left and right eyes respectively of P28 mice. Coronal sections were taken through the dLGN and the distribution of label quantified. Retrograde tracing from the dLGN to the retina was also performed on adult mice. **Results:** Ten-m4 heterozygotes (n=4 dLGNs) were indistinguishable from Ten-m4 KOs with an expansion of anterograde label in caudal dLGN, but differed significantly from wildtyes (WTs, n=12 dLGNs, $p<0.05$, multivariate ANOVA). Surprisingly, Ten-m2 heterozygotes had the same phenotype as Ten-m4 mutants (n=4 dLGNs, $p<0.05$). Double heterozygotes, and Ten-m2/Ten-m4 double knockouts (n=4 dLGNs each) all showed increased ipsilateral label in caudal dLGN relative to WTs. Preliminary retrograde tracing shows an increased ipsilateral projection from the retina consistent with the increased dLGN label. **Conclusion:** All these mice show a phenotype consistent with an increased projection from temporal retina as seen in Ten-m4 knockouts. This suggests a dominant role for Ten-m4. It further suggests a complex interaction between Ten-m2 and Ten-m4, potentially via gene expression, protein-protein or cell surface interactions.

POS-TUE-017

PULSED MAGNETIC FIELDS INDUCE COLLATERAL REINNERVATION IN THE ADULT MOUSE CEREBELLUM

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Purpose: Spontaneous collateral reinnervation of the olivocerebellar pathway in the developing nervous system restores function following neurotrauma, but is lost with age. Injecting brain derived neurotrophic factor (BDNF) into the brain can induce collateral reinnervation in the adult but is invasive. Pulsed magnetic fields (PMF), a non-invasive form of brain stimulation, can up regulate levels of BDNF. This study examined whether PMF can induce collateral reinnervation and improve motor function in adult mice following a unilateral lesion to the olivocerebellar pathway (pedunculotomy). **Methods:**3 month old C57/Bl/6J mice received 10 minutes of sham (n=9) or PMF (n=10) treatment for 14 days, following a unilateral pedunculotomy. VGLUT2 immunohistochemistry was used to quantify and map reinnervation following treatment. Rotarod and hanging wire tasks were used to assess differences in motor function for 4 days following last treatment. A separate group of intact mice received sham (n=6) or PMF (n=6) stimulation for BDNF ELISA analysis to quantify changes in cerebellar BDNF following a single stimulation. **Results:**PMF treated mice had a mean collateral reinnervation of 11.6% whilst sham treated had no reinnervation. No significant difference between treatments was found on the rotarod or hanging wire at this time point. PMF stimulation increased cerebellar BDNF by 160% ($p<0.05$) relative to sham. **Conclusions:**PMF stimulation is a non-invasive technique that can up regulate BDNF and induce collateral reinnervation in the adult mouse cerebellum. Future studies will look at the therapeutic potential of combing PMF and exercise to increase motor improvement.

POS-TUE-018

IN VIVO PROPERTIES OF NEURAL GRAFTS GENERATED FROM HUMAN EMBRYONIC STEM CELLS

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Purpose: Although it has been highly anticipated that stem cells will provide new therapies for brain repair, the reality is that we are still a long way from realising this goal. Most notably, there is still very little information of the capacity for specific types of neurons generated from stem cells to appropriately reconstruct corresponding neuronal pathways after transplantation. We have sought to address this by using a human embryonic stem (ES) cell line expressing green fluorescent protein (GFP) in order to perform detailed neuroanatomical and functional analyses in a series of neural grafting studies. **Methods:** The human ES cell 'Envy' was patterned into neural progenitors by growth on a PA6 feeder layer supplemented with Noggin for 10 days, followed by differentiation as neurospheres for a further 7 days. $1x10^5$ cells were grafted into the striatum or cortex of neonatal or adult rats (n=5/group). Animals were taken for histology or electrophysiological analysis 10 weeks later. **Results:** The grafts were heterogenous in composition, containing around 50% neurons (NeuN+), as well as differentiated astrocytes and oligodendrocytes. The most striking feature was the long-distance outgrowth of axonal fibres along white-matter tracts of the host brain. The innervation patterns were consistent with cortical projection neuron identity, including growth along cortico-cortical, cortico-thalamic and cortico-bulbar pathways. Patch-clamping of grafted neurons showed the generation of action potentials and evidence of functional afferent input onto grafted cells **Conclusion:** These studies show that neurons generated from human ES cells are capable of extensive structural and functional connectivity after transplantation, with predictable patterns of fibre outgrowth. The use of GFP to study neuroanatomical integration in detail will provide an important platform against which to correlate specific aspects of graft integration with functional outcomes in animal models of brain damage.

POS-TUE-019

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

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Purpose: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by reduced cognitive function. Stem cell based approaches are a potential therapeutic option. In order to investigate this possibility, this work shows characterization of a dual reporter embryonic stem (ES) cell line and validation of an immunotoxin mouse model of AD for future implantation experiments.**Methods:** A dual (mcherry and Lhx8+) reporter ES cell line was derived from E14Tg2a mouse ES cell line. The ES cells were assessed for their differentiation capability and characterized using immunohistochemistry. For the immunotoxin model, 6-8 week old C57BL/6 male mice (n = 12) were treated with bilateral intracerebroventricular injections of saline (control) or mu-p75-saporin toxin (0.4µg/µl/mouse) to cause cholinergic neuronal lesions. The mice were cognitively assessed using a novel three day water maze (WM) protocol and the novel object recognition (NOR) paradigm. **Results and conclusions:** A significant difference in learning the WM task was observed during cued and spatial trials, with toxin-treated mice taking longer to reach the platform than control mice (two way ANOVA; $p<0.01$). Performance on the probe trial was also significantly reduced in toxin-treated mice, compared to control mice (t-test; $p<0.05$), indicating memory loss in toxin-injected mice and better learning in the saline-treated controls. However, no memory impairments were detected using the NOR paradigm. Immunohistochemistry for choline acetyltransferase confirmed the loss of cholinergic neurons. These data indicate that the toxin model is appropriate for use in subsequent implantation studies. A slow differentiation capability was observed in the reporter line as compared with wild type cells, a finding to be investigated further in order to select cholinergic progenitors for implantation.

POS-TUE-020

COMPROMISED DEVELOPMENT OF THE CEREBELLUM FOLLOWING INTRAUTERINE GROWTH RESTRICTION

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Purpose: Intrauterine growth restriction (IUGR) leads to adverse neurodevelopmental sequelae postnatally. In IUGR guinea pigs, we have previously demonstrated reduced volumes of the internal granular layer (IGL) and cerebellar cortex postnatally, suggesting impaired granule cell development. Here we investigate the possible mechanisms underlying these alterations in cerebellar development following IUGR. **Methods**: At 30 days of gestation (dg; term ~67 dg), IUGR fetuses were produced by restricting fetal blood flow to one side of the pregnant guinea pig uterus; controls were from sham-operations. At 52dg (n=8 control, n=7 IUGR) and 60dg (n=8 control, n=8 IUGR), cerebellar sections were immunostained to identify proliferating cells (Ki67), post-mitotic cells (p27) and Bergmann glia (GFAP; 60dg only). **Results:** At 52dg, there was no difference ($p>0.05$) in EGL thickness or IGL area in IUGR versus control fetuses. In IUGR fetuses at 60dg, EGL thickness was greater ($p<0.005$) and IGL volume smaller ($p<0.05$) than controls. In the EGL at both ages, there was no difference ($p>0.05$) in the proportion of Ki67-immunoreactive (IR) cells to total cell number. The staining pattern of p27-IR in the EGL was the inverse of Ki67-IR at both ages. At 60dg, there was no difference ($p>0.05$) in the linear density of Bergmann glia between groups. **Conclusion**: EGL thickness in IUGR fetuses at 60dg was comparable to the thickness in fetuses at 52dg, indicating a delayed EGL development. Proliferating granule cells exit the cell cycle, are equipped with a migratory glial scaffold but are unable to populate the IGL. This may be due to apoptosis or altered migratory cues; these possibilities are being assessed.

POS-TUE-021

TEN-M3 IS AN IMPORTANT REGULATOR OF THALAMOSTRIATAL CONNECTIVITY

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Purpose: Ten-m3 is a transmembrane glycoprotein which regulates cell adhesion and axonal guidance during development of the visual system. Here, we investigate a role for Ten-m3 in striatal connectivity. **Methods/Results:** *In situ* hybridisation reveals that Ten-m3 is expressed in a high-low dorsomediocaudal-ventrolaterorostral gradient in the developing striatum. Within this graded expression pattern are patches devoid of Ten-m3. Dual staining with tyrosine hydroxylase to mark striosomes reveals a complementary relationship, localising Ten-m3 to the striatal matrix compartment. A survey of afferent structures further showed Ten-m3 expression in the parafascicular nucleus (PF), a thalamic structure with terminals organised into patches within the striatal matrix, where it is expressed in a high-dorsal to low-ventral gradient, corresponding topographically with the graded pattern within the striatum. Anterograde tracing in adult wild type (WT) and Ten-m3 knock out (KO) mice using biotinylated-dextran amine into the PF show that thalamostriatal terminals fill a significantly larger striatal area in KOs than in WTs ($p=0.030$, t-test, n=8). Further, terminals appear more diffuse and uniform throughout the matrix in KOs. Additionally, tracing experiments using DiI crystals in fixed prenatal brains suggest that thalamostriatal axons first reach the striatum at embryonic day (E)17, with terminals appearing more disorganised and filling a significantly expanded striatal area in KO pups compared to WTs ($p=0.0057$, t-test, n=5) by postnatal day (P)3, consistent with the adult phenotype. Preliminary studies suggest that Ten-m3 may act by altering EphA7 expression, as Western blots suggest that EphA7 protein levels are reduced in Ten-m3 KO striatum. **Conclusion:** Together, these data suggest an important role for Ten-m3 in the guidance and targeting of thalamostriatal afferents.

POS-TUE-022

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 EXPRESSION IN NEUROGENIC ZONES IN DEVELOPING RAT BRAIN

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The VEGF family, known to have a role in neuroproliferation and neuroprotection, acts via receptors VEGFR1-3. VEGFA ligand acting via VEGFR1 is believed to negatively modulate VEGFR2 signalling in neurogenesis. In contrast, VEGFB ligand which preferentially binds VEGFR1 is reported to promote neurogenesis. VEGFR1 mRNA expression has been reported in immature rat brain but its protein expression has not been comprehensively reported in developing CNS. **Purpose:** We examined the cellular expression of VEGFR1 in developing rat forebrain by immunohistochemistry, and compared the pattern to that of VEGFA and VEGFB. We were particularly interested in VEGFR1 expression in neurogenic brain regions. **Methods:** Brains from embryos (E13, E16, E18) and neonates (P2, P7, P15, P23) were fixed and paraffin-embedded. Double-labelling immunohistochemistry was performed with VEGFR1 and nestin, GFAP, NeuN, BT-III, NG2, nNOS, VEGFA and VEGFB using ALEXA secondaries (n=2/age). **Results:** VEGFR1 was highly expressed from E13 in the ventricular zone (VZ) associated with radial glia and the rostral migratory stream. Co-expression with nestin (neural progenitor marker) was found in early development. At later developmental ages, overall VEGFR1 expression and its co-association with nestin-labelled progenitors declined, but a greater association with neurones was found. VEGFR1 was also expressed by oligodendroglial progenitors. VEGFB, but not VEGFA, was detected in the VZ in early development. **Conclusion:** Expression of VEGFR1 by neural progenitors and the presence of its specific ligand VEGFB in the proliferative zones of immature brain support a role in early neurogenesis. With further development, the waning of VEGFR1 expression by neural progenitors and increase by mature neurones is consistent with VEGFR1 having an ongoing but different function in postnatal brain.

POS-TUE-023

FUNCTIONAL ANALYSIS OF HUMAN ESC MIDBRAIN DOPAMINERGIC NEURONS DURING DIFFERENTIATION

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Purpose: Neurons derived from human embryonic stem cells (hESCs) represent an inexhaustible source of cells for transplantation in neurodegenerative disease, for modelling cell physiology and for drug discovery programs. In Parkinson's disease (PD), midbrain dopaminergic neurons of the A9 group are progressively lost which leads to the classic motor symptoms seen. Our lab recently created a human stem cell reporter line expressing eGFP under the control of the specific midbrain dopaminergic transcription factor, PITX3, allowing us to identify these neurons as they differentiate. Given the relatively poor understanding of the functional capabilities of hESC-derived neurons, we asked whether our PITX3-eGFP+ cells responded to common neurotransmitters present in the midbrain, and whether these responses changed during differentiation. **Methods:** Cells were grown in monolayer and imaged with live-cell calcium and chloride ion fluorescence on days 20, 40, 60, and 80 of differentiation. **Results:** At day 20 of differentiation, PITX3-eGFP+ neurons responded to γ-amino butyric acid (GABA), noradrenaline (NA), L-glutamate (Glut), acetylcholine (ACh; all 30μM), adenosine triphosphate (ATP; 300μM), KCl (30mM), and Ca^{2+} (20mM) with elevations of $[Ca^{2+}]_i$. The magnitude of Glut, ATP, KCl, and Ca^{2+} induced elevations increased significantly by day 80, at the same time as GABA induced elevations decreased, indicating functional development during differentiation ($P<0.05$, n=4, 40-60 cells). From day 20 to 80 basal $[Ca^{2+}]_i$ increased from 44.57 ± 12.99 nM to 168.67 ± 25.2 nM, while basal $[Cl^-]_i$ decreased from 5.70 ± 0.35 mM to 2.18 ± 0.39 mM. **Conclusion:** Our results show that PITX3-eGFP+ neurons derived from hESCs recapitulate some key functional aspects of midbrain dopaminergic neurons. Interestingly, the continuing functional development of these cells after the appearance of PITX3 may have implications for the optimal timing of their use in transplantation or basic research.

POS-TUE-024

EPHRIN-A2/A5 IS NECESSARY FOR FUNCTIONAL TOPOGRAPHIC MAP FORMATION IN THE IPSILATERAL RETINOCOLLICULAR PATHWAY

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Purpose: Stereoscopic depth perception requires the integration of binocular inputs. This occurs mainly in the primary visual cortex, but also in the superior colliculus (SC). In mice, the vast majority of output from the retina is to the contralateral SC; only 1-3% of retinal ganglion cells project ipsilaterally. Both projections are topographically ordered, but the ipsilateral map is reversed relative to the contralateral one in order to maintain visuotopy. Topographic order of the contralateral projection is established during development through mechanisms including ephrin-A guidance ligands, plus patterned retinal activity and visual experience, but less is known about the mechanisms that guide the reversed ipsilateral projection. We used monocular enucleation to investigate the cues involved in forming the ipsilateral retinocollicular map. **Methods:** Ephrin-A2/A5 double knockout mice (KO; n=7) and wildtype (WT; n=7) mice had one eye removed on postnatal day 1 and topography of the ipsilateral retinotectal projection was mapped by multiunit electrophysiological recording in the SC when adult. **Results:** Following enucleation, the ipsilateral projection in WT mice mapped appropriately, demonstrating visuotopy. However, 100% of KO mice had inaccurate maps: 72% had maps with rostral-caudal disorder in which topography is reversed such that temporal retina mapped to rostral SC. 57% additionally displayed medial-lateral disorder. Duplicate maps were recorded in 14% of KO mice, with multiple ectopic terminations (4 from 17 recorded sites). **Conclusion:** Our results demonstrate that in the absence of contralateral input, the ipsilateral retinotectal projection requires ephrin-A2 and/or ephrin-A5 to establish the visuotopic reversed topographic projection. We suggest that ipsilaterally and contralaterally projecting retinal axons respond differently to ephrin-As.

POS-TUE-025

CHANGES IN THE EXPRESSION OF BDNF AND TRKB IN THE HIPPOCAMPUS DURING ADOLESCENCE IN C57BL/6 MICE

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Purpose: Brain-derived neurotrophic factor (BDNF) is implicated in schizophrenia which shows sex differences and tends to emerge during adolescence. We therefore investigated levels of BDNF and its receptor, TrkB, from pre-pubescence to adulthood in male and female C57Bl/6 mice. **Methods:** BDNF and TrkB expression were assessed by Western blot and fluorescent immunohistochemistry and compared to serum estradiol and testosterone levels. First, a week by week analysis was conducted in dorsal (DHP) and ventral (VHP) hippocampus (n=5-6/week). Subsequently, gonadectomy and hormone replacements were done at 5 weeks of age followed by BDNF/TrkB analysis at 8-9 weeks (n=8 9/treatment). **Results:** Females showed significant age-related changes in BDNF and TrkB phosphorylation with levels peaking at week 6. Immunohistochemistry revealed highest BDNF expression in the CA3 sub-region of the DHP followed by DG, CA2 and CA1. Staining intensity dramatically increased from week 4 to 5, particularly in the hilar region and CA3, and gradually decreased from week 6 to adulthood. These changes did not correspond with serum estradiol levels and ovariectomy and estradiol replacement had no effect. Male mice showed no significant changes in BDNF-TrkB signalling during adolescence despite a significant peak in serum testosterone levels, and no effect of castration was found. **Conclusion:** These results demonstrate significant adolescent changes in BDNF-TrkB signalling across discrete regions of the hippocampus in female, but not male mice. The differential role of sex steroid hormones in modulating these changes remains unclear. Our approach may help to identify critical developmental windows, at a sub-region level, for intervention in neurodevelopmental psychiatric disorders.

POS-TUE-026

A PEPTIDE MIMETIC OF BDNF PROMOTES PERIPHERAL MYELIN DEVELOPMENT AND REPAIR

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The neurotrophins are essential for peripheral nervous system development and myelination. We have previously demonstrated that the neurotrophin BDNF exerts contrasting influences upon myelination - acting through neuronal p75NTR to enhance myelination, but inhibiting it via neuronal TrkB. We have generated a small peptide called cyclo-DPAKKR that structurally mimics the region of BDNF that binds p75NTR. **Purpose**: Here we aim to investigate whether utilising cyclo-DPAKKR to selectively target p75NTR is an approach that could exert a unified promyelinating response. **Results**: Like BDNF, cyclo-DPAKKR promoted myelination of NGF-dependent neurons *in vitro*, an effect dependent on the neuronal expression of p75NTR. Importantly, whereas BDNF inhibited the myelination of BDNF-dependent neurons *in vitro*, cyclo-DPAKKR significantly enhanced it (n=3). Local injection of cyclo-DPAKKR adjacent to the neonatal sciatic nerve *in vivo* significantly enhanced myelin protein expression and increased the number of myelinated axons (n=6). We found that injection of cyclo-DPAKKR also significantly upregulated the expression of Neuregulin 1 type-III, a key factor required to induce peripheral myelination. Furthermore, administration of cyclo-DPAKKR caused a delay in the onset of clinical disability in experimental autoimmune neuritis, a mouse model of peripheral nerve demyelination and significantly reduced the clinical disease severity (n=6). We are currently characterising the interaction between cyclo-DPAKKR and p75NTR at the molecular level using the NMR spectroscopy technique. **Conclusion**: These results demonstrate that using cyclo-DPAKKR to selectively target p75NTR promotes peripheral myelination *in vitro* and *in vivo*, and importantly delays the onset and reduces the severity of a mouse model of peripheral nerve demyelination. Our findings suggest that selective targeting of p75NTR is a strategy worthy of further investigation for the treatment of peripheral demyelinating diseases.

POS-TUE-027

THE EFFECTS OF HUMAN AMNION EPITHELIAL CELLS (hAECs) FOLLOWING FETAL INFLAMMATION

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Purpose: Intrauterine inflammation is recognized as a major cause of preterm birth and neurological complications in the developing brain. This study aims to examine whether human Amnion Epithelial Cells (hAECs) can be used as a potential therapeutic agent to reduce brain injury induced by inflammation (lipopolysaccharide, LPS) in preterm fetal sheep. **Methods:** Pregnant ewes underwent surgery at ~105 days of gestation for implantation of catheters into the fetal brachial artery and femoral vein. LPS was administered at 109, 110 and 111 d via the femoral vein and fluorescent-labeled hAECs were administered at 110, 111, and 112 d via the brachial artery. Control fetuses received saline. Brains were collected at 114 d gestation for histological assessment of brain injury. **Results:** hAECs were observed throughout the brain, with large numbers (up to 30 ± 5 cells/mm^2) identified in the white matter, cortex and the hippocampus. Pyknotic degenerating cells were evident and increased within LPS brains; in the thalamus (100-fold increase vs control), and hippocampal CA1 region (80-fold increase vs control). hAECs administration significantly reduced pyknotic cell numbers in the thalamus and the hippocampus compared to LPS alone. The number of neurons within the CA1 region of the hippocampus was significantly reduced in the LPS brains (57 ± 20 cells/mm^2) compared to control and LPS+hAECs (761 ± 13 cells/mm^2) -treated animals. Circulating fetal cytokine concentrations have been evaluated and it appears that hAECs reduce TNF-α levels 6 hours following LPS administration. **Conclusion:** hAEC administration to fetuses in an LPS model of fetal inflammation reduces neuronal cell loss, which is likely mediated by dampening the fetal inflammatory response.

POS-TUE-028

CELL CYCLE EXIT STUDIES OF DIFFERENT NEURON SUBTYPES IN THE MOUSE SMALL INTESTINE

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Purpose: There are many different functional types of enteric neurons. A landmark study by Pham et al (J Comp Neurol 314:789-798, 1991), which used tritiated thymidine birthdating, first showed that different enteric neuron subtypes in the mouse differ in their time of exit from the cell cycle. Although myenteric neuron subtypes in the mouse have been well characterized based on neurochemistry, the peak times of cell cycle exit for some major enteric neuron subtypes are still incompletely characterized or unknown. The age at which cell cycle exit occurs is an important determinant in the differential response of different subtypes of enteric neurons to developmental cues and disturbances. **Methods:** Time plug-mated C57/Bl6 mice received a single intraperitoneal injection of EDU at E10.5, E11.5, E12.5, E13.5, E15.5 or E18.0. P0 and P10 mice also received a single intraperitoneal injection of EDU. Except for tissue processed for CGRP immunohistochemistry, the mice were killed at 5-8 weeks of age by cervical dislocation, and the small intestine removed and processed for immunohistochemisty using antibodies to calretinin, CGRP, 5-HT, TH, NOS and NF-M. **Results:** The order of cell cycle exit was 5-HT neurons (peak exit at E11.5), CGRP and NF-M neurons (peak exit at E12.5-E13.5), TH neurons (peak exit at E15.5), NOS neurons (peak exit at E15.5) and calretinin neurons (peak exit at P0). Although NOS neurons are one of the first types of neurons to appear, and are already present in the E11.5 gut (Hao et al., Neurogasto Motil 22:e127-37), we did not observe any NOS neurons that had incorporated EDU following EDU injections at E10.5 or E11.5. **Conclusion:** As in other parts of the nervous system, different functional classes of neurons exit the cell cycle at different ages.

POS-TUE-029

TEN-M2 IS REQUIRED FOR THE GENERATION OF BINOCULAR VISUAL CIRCUITS

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Purpose: Mechanisms that lead to the stereotyped organisation and integration of binocular inputs within the brain are critical for visual function. Previous experiments have indicated a role for the transmembrane glycoprotein, Ten-m3, in mapping of ipsilateral retinal projections. Here, we investigated the role of a closely-related Teneurin/Odz/Ten-m family member, Ten-m2, in the formation of ipsilateral projections in the mouse visual system. **Methods and Results:** Anterograde and retrograde tracing in Ten-m2 knockout (KO) mice revealed a reduction in ipsilateral projections from the retina that was more prominent in ventral regions ($p<0.05$, t-test) with a corresponding expansion of contralateral projections ($p<0.05$, t-test). More subtle changes were also observed in temporal retina ($p<0.01$, t-test). While expression of a critical ipsilateral fate determinant, Zic2, appeared unaltered, in situ hybridisation revealed a notable reduction in one of its downstream targets, EphB1, in ventral retina of KOs ($p<0.05$, t-test), suggesting that Ten-m2 acts within this molecular pathway. Immunohistochemistry for c-fos, a marker for neural activity, revealed that the area of primary visual cortex (V1) driven by ipsilateral inputs was reduced in KOs ($p<0.05$, t-test). Further, the ratio of ipsilateral-to-contralateral responses contributing to binocular activation during visually-evoked potential (VEP) recordings were also diminished ($p<0.05$, t-test). Finally, a novel visual discrimination task demonstrated a specific impairment of KOs to discriminate between dorsally-located visual stimuli ($p<0.005$, Mann-Whitney U-test), consistent with both the ventral retinal deficit and VEP data. **Conclusion:** Together, these data highlight the requirement of Ten-m2 in formation of the ipsilateral projection and generation of functional binocular circuits.

POS-TUE-030

HETEROGENEITY OF ENS CELL FATE REVEALED BY CLONE-LABELLING IN SITU AND MATHEMATICAL MODELLING

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Purpose: The role of enteric neural crest cells (ENCCs) in enteric nervous system (ENS) development has been studied at the population level. This project focuses on ENCC migration, proliferation and differentiation in the intestine at the individual cell level. Methods: Hindbrain quail embryo pre-migratory NCCs were electroporated with GFP plasmid at E1.5, then at E3.5 a fragment of foregut containing one GFP+ ENCC was isolated and combined with E4 ENCC wavefront. This was fused to E4 aneural gut and the combination was grown in vitro for 4 days (minimal gut growth) or 8 days in CAM graft culture (normal gut growth) to allow colonisation. Results were analysed by cell counts of ENCCs and neurons in wholemounts. Cellular automaton (CA) models encoded movement, proliferation, differentiation, and gut growth. Results: Single ENCC gave rise to 1 to >2000 progeny. In guts that developed numerous GFP+ cells, these cells formed multiple loose groups of unpredictable cell number and placement. The GFP+ cells were always mixed with GFP-ve cells. However where GFP+ cell density was high (>25% of local ENS), most GFP+ cells were not neurons, while in small groups of GFP+ cells, many cells were neurons. CA models encoded stochastic cell movement, stochastically placed but logistically limited proliferation and differentiation, according to our previous biological observations. From these emerged in silico a stereotypes pattern of ENS development at cell population level but extraordinary variability in proliferation, distribution and differentiation at the individual cell level. Conclusion: The self-organising principles of the ENS are populational with huge diversity at the level of individual cells.

POS-TUE-031

DIFFERENTIAL EFFECTS ON DYNAMIN I GTPASE ACTIVITY OF GST TAGGED OR UNTAGGED SH3 DOMAIN PROTEINS

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Purpose: Dynamin I is a GTPase enzyme required for synaptic vesicle endocytosis (SVE). During SVE dynamin oligomerises, resulting in greatly increased GTPase activity. Dynamin interacts with various SH3 domain containing proteins during endocytosis. Only three SH3 domains are known to stimulate dynamin despite >20 are being to bind dynamin. We aimed to investigate the effect of a variety of SH3 domains on GTPase activity. Previous studies used SH3 constructs containing the GST expression and purification tag, which is a dimer with potential to crosslink artefactually dynamin via the SH3 peptides. We therefore also investigated the effect of this tag. **Method:** The SH3 domains of 8 proteins were recombinantly expressed and purified. GST tag was removed by on-bead digestion with thrombin or precision proteases. SH3 proteins with and without the GST tag, at a range of concentrations, were incubated with dynamin I. GTPase activity was determined by a colourimetric assay (n=3). A pull-down of dynamin from rat brain by SH3 proteins was performed to determine the relative binding of each SH3 domain to dynamin. **Results:** The pull down showed that all 8 SH3 domains bound the same absolute amount of dynamin. All 8 GST tagged SH3 domains stimulated dynamin, but in contrast to the binding study, each stimulated GTPase activity to markedly different extents. GST-tagged proteins were generally more potent than untagged SH3s. The notable exception was SNX9 SH3, which stimulated dynamin GTPase activity to a greater extent in the absence of the tag. **Conclusion:** Dynamin GTPase activity is stimulated by a wide array of SH3 domains, but to surprisingly varied extents. The very high stimulation of dynamin activity by GST cleaved SNX9 SH3 domain demonstrates that the GST tag is not a generic crosslinker. The observations suggest high specificity in regulation of dynamin activity by SH3 domains.

POS-TUE-032

FUNCTIONAL ROLE OF DEVELOPMENTALLY REGULATED ALTERNATIVE SPLICING OF A SODIUM CHANNEL GENE

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Purpose: The $Na_v1.2$ sodium channel alpha subunits have two developmentally regulated splice variants; the 'neonatal' and 'adult' isoforms. Previous studies from our laboratory have demonstrated that the 'neonatal' isoform is less excitable than the 'adult'; moreover, a mutation discovered in patients with benign familial neonatal-infantile epilepsy (BFNIE) increases the excitability of the 'neonatal' isoform such that it resembles the adult isoform. We hypothesize that the physiological role of 'neonatal' $Na_v1.2$ is to reduce neuronal excitability in infant brains. To test this we have engineered a mouse line which only expresses 'adult' $Na_v1.2$ and investigated seizure susceptibility and neuronal phenotypes. **Methods:** Whole-cell patch clamp was used to compare electrophysiological properties in cortical layer 2/3 pyramidal neurons of 3 day old wild-type and homozygous 'adult' $Na_v1.2$ mice. Current clamp protocols utilizing episodic stimulation were used to measure input-output relationships. Seizure susceptibility was determined by administering subcutaneous pro-convulsant pentylenetetrazol (PTZ, 120mg.kg-1) to 45 day old mice and time to hind limb extension was recorded. **Results:** Electrophysiological analysis revealed that both wild-type and 'adult' $Na_v1.2$ neurons displayed a range of firing patterns presumably due to different levels of maturity. Broadly, neurons could be classified into low-firing and normal-firing classes. For the normal-firing class 'adult' $Na_v1.2$ neurons (n=5) displayed increased firing compared to the wild-type, consistent with hyperexcitability. Furthermore, homozygous 'adult' $Na_v1.2$ mice had an increased susceptibility to PTZ-induced seizures compared to wild-type littermates (n=8, $p<0.05$) consistent with the idea that the 'neonatal' $Na_v1.2$ confers protection against hyperexcitability. **Conclusion:** 'Adult' $Na_v1.2$ neuron hyperexcitabilty and increased seizure susceptibility suggests that 'neonatal' $Na_v1.2$ may confer a degree of seizure protection during development.

POS-TUE-033

SEIZURE-RELATED GENE 6 ACTIVATES CALCIUM-DEPENDENT SIGNALLING IN DEVELOPING NEURONS

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PURPOSE: Seizure-related gene 6 (Sez-6) is required for the normal development of cortical pyramidal neurons. Mature neurons in Sez-6 knockout (KO) mice have excessive dendritic branching and fewer excitatory synapses. *In vitro* experiments indicate that Sez-6 stabilises dynamic filopodia during dendritogenesis. Since transient changes in intracellular calcium levels ($[Ca^{2+}]_i$) and activation of calcium-dependent signalling pathways are associated with the stabilisation of dendritic filopodia, we examined the influence of Sez-6 on neuronal calcium dynamics. **METHODS:** Changes in $[Ca^{2+}]_i$ were measured in cultured mouse cortical neurons, loaded with the calcium indicator dye Fluo4-AM and perfused with culture medium containing soluble Sez-6 type III. Western blotting was used to examine activation of calcium-dependent pathways in cultured neurons and brain slices treated with Sez-6 type III protein, and in the cortex and hippocampus of Sez-6 KO and wild-type (WT) mice. **RESULTS:** Cortical neurons perfused with Sez-6 type III conditioned medium displayed an elevation in cytoplasmic $[Ca^{2+}]_i$ and increased phosphorylation of calcium/calmodulin-dependent kinase II (CaMKII); both changes were partially blocked by pre-incubation with the voltage-sensitive calcium channel inhibitors nimodipine and ω-conotoxin MVIIC. Preliminary data indicates treatment with Sez-6 type III protein also alters cytoplasmic $[Ca^{2+}]_i$. Treatment of brain slices with Sez-6 type III protein activated Erks 1, 2 and 5, key enzymes in calcium-dependent signalling pathways. Lower levels of autoactivated CaMKII were detected in Sez-6 KO compared to WT brains, suggesting Sez-6 expression alters calcium dynamics in vivo. **CONCLUSIONS:** Sez-6 may facilitate dendritic development and synaptogenesis by modulating calcium signalling to stabilise dendritic filopodia.

POS-TUE-034

AUTOMATED PLANAR PATCH CLAMP REVEALS DIFFERENTIAL MODULATION OF SODIUM CHANNEL AUXILIARY SUBUNITS IN THE BRAIN

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Purpose: Voltage-gated sodium channels are composed of an α subunit, commonly associated with two auxiliary β subunits. As different α-subunits are present in excitatory and inhibitory neurons and in different sub neuronal compartments, it is important to understand the differential effects the β-subunits may have on α-subunits, to assist in understanding how they modulate neuronal function both in health and disease. In the present study we used automated planar patch clamp to investigate the effect of β_1 and β_2 subunit modulation on the "excitatory neuron" α-subunit: $Na_V1.2$, and the "inhibitory interneuron" α-subunit: $Na_V1.1$. **Methods**: Automated patch clamping using the Nanion patchliner, was used analyse α:β subunit combinations transiently transfected into HEK293T cells. **Results**: Analysis of $Na_V1.2$ with the β_1 subunit revealed a hyperpolarising shift in the voltage-dependence of inactivation ($p<0.001$, N=30), larger time constants of inactivation ($p<0.001$, N=18) and a quicker recovery from inactivation ($p<0.001$, N=31). $Na_V1.2$ with β_1 and β_2 revealed only a slower recovery from inactivation ($p<0.01$, N=19). In contrast, when β-subunits were co-expressed with $Na_V1.1$ the only parameter significantly modulated was recovery from inactivation, which was slower ($p<0.001$, N=13), when β_1 and β_2 were co-expressed. However β-subunits did increase current density of $Na_V1.1$, which was not seen with $Na_V1.2$. **Conclusion**: We observed a differential effect of β-subunits on the "excitatory neuron" α-subunit $Na_V1.2$, and the "inhibitory interneuron" α-subunit $Na_V1.1$. If, in the context of disease, a β_1 variant experiences functional change, our results suggest that this will result in differentially altered levels of excitation and inhibition in the brain, the imbalance of which, could feasibly give rise to a disorder of excitability, such as epilepsy.

POS-TUE-035

SEIZURE ASSOCIATED CHANGES IN THE HIPPOCAMPUS OF A KNOCK-IN MOUSE MODEL OF ABSENCE EPILEPSY

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Purpose: Although absence seizures are derived from cortico-thalamic networks,associated co-morbidities may result from hippocampal deficits. DBA mice, heterozygous for the susceptibility mutation $GABA_A\gamma$2R43Q(DBA(R43Q)), are an experimental model of absence epilepsy. Spontaneous spike-and-wave discharges (SWDs) associated with behavioural arrest occur from P24. In contrast, C57(R43Q) mice have no SWDs and act as a seizure-free control. In this study we determine if SWDs engage the hippocampus by analyzing both, expression of the Hyperpolarization-activated-Cyclic-Nucleotide-gated channel HCN1, a channel known to respond to developmental and environmental cues including febrile seizure, and related changes in function. **Methods:** HCN1 mRNA in the hippocampus was assayed by qPCR in seizing and seizure-free mice.The effect on I*h* was measured using whole-cell voltage clamp of CA1 pyramidal cells. Spatial learning was assayed in the Morris Water Maze. (n≥12 in all studies). **Results:** Hippocampal HCN1 was reduced in seizing DBA(R43Q) p=0.0049, but not pre-seizure or, in seizure-free C57(R43Q). HCN1 reduction required >2SWDs/hour but more frequent seizures did not further increase HCN1 reduction. Voltage clamp studies of CA1 pyramidal neurons revealed reduced I*h* in seizing mice. Further, a left-shift in $V_{1/2}$ implied a larger proportion of HCN2 current relative to HCN1. Spatial learning defects, day 5 p=0.041, were apparent in adult DBA(R43Q) but not in C57(R43Q). **Conclusions:** Cortico-thalamic derived SWDs alter HCN1 expression and function in the hippocampus,with accompanying spatial learning deficits. We suggest that HCN1 reduction may be a biomarker for hippocampal-based seizure co-morbidities.

POS-TUE-036

ALTERED SPINE MORPHOLOGY FOLLOWING CALCIUM WAVES IN BASOLATERAL AMYGDALA PROJECTION NEURONS

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Purpose: Connections between excitatory neurons primarily occur on spines. Spines are linked to the dendrite via a thin spine neck that restricts diffusion between the two compartments. This arrangement concentrates calcium entering through NMDA receptors in the in spine head facilitating the induction of long-term synaptic potentiation by high frequency stimulation (HFS) at the stimulated synaptic inputs. In projection neurons in the basolateral amygdala (BLA), HFS also activates metabotropic receptors and evokes a focal rise in dendritic calcium that propagates as a wave along the dendrite. Here we investigated whether passing calcium waves invade spines and alter their synaptic connections. **Methods:** Brain slices were prepared from Wistar rats (21-35 d) anesthetized with isoflurane, and killed by decapitation. Whole-cell patch-clamp recordings and two-photon fluorescence images were made from BLA projection neurons loaded with the calcium indicator Fluo5F and the calcium insensitive dye Alexa 594. **Results:** Calcium waves evoked by HFS differentially invaded spines as they propagated along the dendrite, preferentially invading those with short necks. In spines with short necks, repetitive bouts of HFS (5 at 60s intervals) resulted in a reduction in the spine head volume (87 ± 6 % of baseline; n = 9) 10 minutes after the first wave. The volume of long-necked spines, which are shielded from dendritic waves, was unchanged (104 ± 6 % of baseline). Spines located on dendrites where no calcium waves were evoked were also unchanged (102 ± 2 % of baseline) following HFS (n= 52). **Conclusion:** Changes in synaptic strength are known to be accompanied by concomitant changes in spine head volume with decreased synaptic strength being accompanied by a reduction in the spine head volume. As calcium waves preferentially invade spines with short necks these results suggest that calcium wave invasion depresses the strength of unstimulated inputs.

POS-TUE-037

SUBTYPE SELECTIVE MODULATION OF HCN CHANNELS BY ANTIEPILEPTIC DRUGS

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Purpose: HCN1 and HCN2 have been implicated in epilepsy with both genetic variation and transcriptional changes described suggesting that these channels are targets for pharmacological intervention. Lamotrigine and gabapentin are two well-established anti-epileptic drugs and brain slice experiments have suggested that they act by modulating HCN channels. Our aim was to further dissect this action by determining HCN isoform specific pharmacosensitivity using automated medium-throughput high content assay. **Methods:** cDNA's of human HCN1 and HCN2 were transcribed and mRNA injected into oocytes. After 48 hours two-electrodes voltage clamp experiments were performed using the Roboocyte V1 platform. Conductance-voltage (G-V) relationships were constructed from normalized tail currents and fit with a Boltzmann equation to measure $V_{1/2}$ and slope before and after drug application. **Results:** Lamotrigine (50µM) reversibly left-shifted the GV curve of HCN1 (-74.2±0.6 mV vs -79.5±0.7 mV, p<0.05, n=14) but was without effect on HCN2 channels (p>0.05, n=7). In contrast, gabapentin (100µM) a small reversible right-shift in the G-V curve of HCN2 channels was observed (-68.6±0.7 vs -66.3±1.0, p<0.05, n=20) but was without effect on HCN1 channels (P>0.05, n=7) **Conclusion:** Genetic and pharmacological data strongly supports HCN1 and HCN2 channels as good antiepileptic drug targets. Subtype selectivity and differential modulation may be an important factor for future drug development.

POS-TUE-038

ALTERATION OF GABA-A RECEPTOR SUBUNITS EXPRESSION WITHIN THE THALAMUS OF STARGAZER MOUSE MODEL OF ABSENCE EPILEPSY

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Purpose: Absence seizures are known to arise from disturbances within thalamocortical networks. Reciprocal projections between inhibitory neurons of the reticular thalamic nucleus (RTN) and excitatory relay neurons of the ventral posterior (VP) complex contribute to an intrathalamic oscillatory circuit. Experimental evidence from rodent models suggests region-specific changes in phasic and tonic $GABA_A$ receptor inhibition underlie hypersynchronous oscillations in absence seizures. Phasic inhibition is mediated by synaptic $GABA_A$Rs with different regional subunit compositions. $GABA_A$Rs at synapses in the VP region are pro-oscillatory (α1β2γ2), whereas $GABA_A$Rs at RTN synapses (α3β3γ2) are anti-oscillatory. The aim of this study was to investigate changes in $GABA_A$R expression in the RTN and VP region of the stargazer mouse model of absence epilepsy. **Methods:** Immunofluorescence confocal microscopy and Western blotting were used to investigate changes in the expression of $GABA_A$R subunit α1 and β2 in the VP and α3 subunit in the RTN. **Results:** $GABA_A$R α1 and β2 subunits were exclusively localized to the VP, whereas $GABA_A$R α3 subunit was predominantly expressed in the RTN. Western blot analyses of samples from RTN and VP regions showed that $GABA_A$R subunits in the VP were upregulated in epileptic mice (α1: 33% increase, n=8, p<0.05; β2: 110% increase, n=3, p<0.01), while expression of α3 remained unchanged in the RTN (n=8, p>0.05). **Conclusions:** These results suggest region-specific changes in $GABA_A$R subunits in the thalamus of epileptic mice. Upregulation of phasic $GABA_A$Rs in the thalamic relay nuclei may contribute to generation of hypersynchronous thalamocortical activity in absence seizures. Understanding region-specific changes in $GABA_A$R expression could be important for development of more specific and effective drugs for absence epilepsy.

POS-TUE-039

STUDY OF THE DISRUPTION OF NERVE-CELL CONNECTIONS IN ALZHEIMERS DISEASE THROUGH THE ASSAY OF TRANS-SYNAPTIC PROTEIN NEUROLIGIN1 AND 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 synaptic damage, and ultimately to neuronal death via glutamate-mediated excitotoxicity. **Aim:** To investigate the disruption of nerve-cell connections in Alzheimer's disease through the assay of the trans-synaptic proteins Neuroligin 1 and Neuroligin 2 and to correlate them with the pathological severity of the disease. Methodology: Neuroligin-1 and Neuroligin-2 proteins were 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-1 and neuroligin-2 standards. **Results:** Area based analysis showed that neuroligin1 and neuroligin2 proteins levels in occipital cortex and inferior temporal cortex did not differ between cases and controls. The level of neuroligin 1 in hippocampus was significantly lower in AD cases (35 ng/mg of total protein) than in sex-and age- matched controls (20 ng/mg of total protein). Correspondingly, the neuroligin 2 level in hippocampus was significantly higher in AD cases (60 ng/mg of total protein) than age-and sex- matched controls (20 ng/mg of total protein). **Conclusion:** The fluctuations of NLGN1 and NLGN2 levels in hippocampus could underpin excitatory and inhibitory synaptic dysfunctions that might leads to excitotoxicity.

POS-TUE-040

ACTIVE DENDRITIC INTEGRATION IN DIRECTION-SELECTIVE RETINAL GANGLION CELLS

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Active dendritic synaptic integration has been reported in several classes of central neuron. A direct role of active dendritic integration in the generation of neuronal computations in intact neuronal circuits has however not been described. Here we investigate the role of dendritic integration in the formation of a neuronal computation in retinal ganglion cells maintained in an isolated and intact preparation of the mammalian retina. We made simultaneous whole-cell somatic and dendritic patch-clamp recordings (n = 44) from ON-direction selective retinal ganglion cells in dark-adapted rabbit retina maintained ex-vivo. Dendritic spikes could be readily evoked by direct current-injection through the dendritic recording electrode, and forward propagated to the axon to initiate action potential firing. The local application of the sodium channel blocker TTX (1 µM) abolished dendritic spikes and attenuated back-propagating action potentials (n = 2). Dendritic spikes were evoked in response to physiological stimuli during the movement of a light bar across the retina in the preferred direction (240 µm/s, 400 x 200 µm), when the light bar crossed the recorded dendritic subfield. Light evoked dendritic spikes were variable in amplitude and their capacity to initiate axonal action potentials (n = 28 recordings 150 to 380 µm from soma), suggesting the existence of multiple dendritic spike initiation zones. The local dendritic application of the GABAA receptor antagonist gabazine significantly enhanced action potential output to null direction light stimuli, and unmasked the generation both large and small amplitude dendritic spikes (n = 4). These data indicate that active dendritic integration directly contributes to the computation of direction selectivity in retinal ganglion cells, through the engagement of multiple integration compartments, which are powerfully controlled by synaptic inhibition.

POS-TUE-041

FUNCTIONAL CONSEQUENCES OF MULTIPLE PHOSPHORYLATION OF CAMKII

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PURPOSE: Calcium/calmodulin-stimulated protein kinase II (CaMKII) is a serine/threonine kinase that controls many processes. CaMKII is controlled by multi-site autophosphorylation and targeting. Autophosphorylation of CaMKII at T286 has been well-characterised, and is involved in several functions, including synaptic plasticity. T286 phosphorylation can induce LTP in the absence of T305/6 phosphorylation, or LTD when T305/6 is also phosphorylated (J Neurosci 30:8704-9), indicating that the effects of T305/6 phosphorylation overrides the effects of T286 phosphorylation. Additionally, double phosphorylation of CaMKII can alter targeting. Phosphorylation of CaMKII at T286 or T253 enhances binding to postsynaptic densities and phosphorylation at both sites gives an additive effect (J Neurochem 79:1122-8). We investigated whether the functional outcomes following double phosphorylation of CaMKII can be predicted based on the outcomes of singly phosphorylated CaMKII. METHOD: MDA-MB-231 cells were transfected with single or double phosphomimic αCaMKII mutants (empty vector, wild-type, T253D, T286D, T305D, T253D/T286D, and T286D/T305D; n=3). Cell proliferation/metabolism and cell cycle progression (resazurin assay/flow cytometry; n=3), CaMKII activity (auto- and exogenous substrate phosphorylation) and targeting (overlay binding assay) following single and double phosphomimic mutation were examined (n=3). RESULTS & CONCLUSIONS: Using cell cycle and proliferative measures in a non-neuronal cell line, we have confirmed that T305 phosphorylation can override the functional consequences of T286 phosphorylation. The mechanisms could involve changes in activity and/or targeting, since both properties were altered by double phosphomimic mutation. By contrast, the T253D/T286D double phosphomimic displayed functional consequences different from either of the single phosphomimics and protein binding, but not kinase activity, was altered suggesting that a change in targeting was responsible for the difference in functional outcome.

POS-TUE-042

IMPACT OF SOMATIC VERSUS DENDRITIC INHIBITION ON NEURONAL OUTPUT

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Purpose: Recent findings in mouse visual cortex have come to opposite conclusions on how interneurons targeting the soma versus dendritic compartments transform the firing output of pyramidal neurons. Here we attempt to resolve this controversy using a modeling approach. **Methods:** We constructed a two-compartment model of a cortical pyramidal neuron. The somatic compartment contained Hodgkin-Huxley-type voltage-gated conductances (Na+, K+, M-type), as well as an after-hyperpolarisation mechanism. The dendritic compartment was modeled as a passive compartment connected to the somatic compartment by a resistor. Input/output relationships were generated by driving the model with depolarizing somatic current or dendritic excitatory synaptic input with and without somatic or dendritic inhibition. We also investigated the impact of random noise modeled by an Ornstein-Uhlenbeck process. All simulations were performed in MATLAB. **Results:** We first studied the impact of inhibition on neuronal output during somatic current injection. Under these conditions, tonic inhibition targeted to the somatic or dendritic compartment had a purely subtractive effect on the input/output relationship. This effect was largely unaltered by injection of random noise. We next tested how tonic inhibition targeted to the somatic or dendritic compartment affected the input/output relationship during excitatory synaptic input to the dendritic compartment. In this case, irrespective of location, inhibition had a more complex effect involving both subtractive and divisive components. The divisive effect was larger for somatic inhibition, and was enhanced when inhibition was recruited in a balanced manner with excitation. **Conclusion:** Our model predicts that the impact of inhibition depends critically on the stimulus used to drive neuronal output, with somatic inhibition more divisive than dendritic inhibition during dendritic excitatory input.

POS-TUE-043

ELECTROPHYSIOLOGICAL PROPERTIES OF CRANIAL AND SPINAL MOTONEURONS IN MICE

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Motoneurons differ in the motor behaviours they control and their vulnerability to disease. For example, cranial motoneurons such as hypoglossal motoneurons (HMs) are involved in licking, suckling, swallowing and vocalization and are often spared in neurological diseases. In contrast, spinal motoneurons (SMs) innervating the limbs are involved in locomotion and are especially vulnerable in motoneuron diseases. Purpose: To compare the electrophysiological properties of HMs and SMs in age-matched mice. Methods: Transverse slices (300 µm thick) were obtained from the brainstem or lumbosacral spinal cord of C57Bl/6 mice (P7-10). Whole-cell recordings were made from visualized motoneurons at 23°C using a KCH_3SO_4-based internal. Results: Intrinsic membrane properties were remarkably similar in HMs and SMs (n = 28 and 26, respectively). No differences were observed in input resistance, cell capacitance and resting membrane potential. In addition, action potential (AP) properties such as rheobase, threshold, amplitude and afterhyperpolarization did not differ between the two populations. In contrast, AP half-width was smaller in HMs (0.95 ± 0.03 vs. 1.49 ± 0.08 ms). HMs discharged at higher frequencies in response to square step (1 s duration, 50 pA increments, 450 pA above rheobase; 31.3 ± 1.3 vs. 24.5 ± 2.5 Hz; n = 27 and 15) and triangular ramp current injection (0.3 nA/s, maximum frequency 42.2 ± 4.0 vs. 26.9 ± 2.4 Hz, n = 17 and 10). Conclusions: HMs and SMs have similar intrinsic properties, however, their discharge in response to similar levels of depolarizing current differs markedly. This suggests each population possess differing suites of ion channels that allow them to undertake their distinct motor functions.

POS-TUE-044

GRIP-PING CHANGES IN SYNAPTIC PROTEIN INTERACTIONS IN STARGAZERS

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Purpose: Glutamate-Receptor-Interacting-Proteins (GRIP1&2) and Protein-Interacting-with-C-Kinase-1 (PICK1) are scaffolding proteins involved in synaptic GluA2/3-AMPA receptor anchorage and recycling. AMPAR phosphorylation causes AMPAR internalisation by decreasing the affinity of GRIP for AMPARs, but not PICK1. Gain-of-function GRIP1 mutations accelerate AMPAR recycling and influence social behaviour in autism. AMPARs are trafficked to synapses by Transmembrane-AMPAR-Regulatory-Proteins (TARPs). Stargazer mice have a TARP-γ2 (stargazin) mutation causing synaptic AMPAR deficits in the cerebellum and thalamus, resulting in ataxia and epilepsy. The aim of this study was to investigate the effects of the stargazer mutation on cerebellar and thalamic GRIP and PICK1 levels in stargazers. **Methods:** GRIP and PICK1 expression were analysed in stargazers and controls by Western-blotting (n=10pairs) and confocal-immunofluorescence (n=3pairs). Quantitative post-embedding immunogold electron-microscopy was used to compare GRIP levels at synapses (n=4pairs, 280 synapses). **Results:** Western-blot analysis showed GRIP expression was significantly increased (70%, $p<0.01$) in stargazer cerebella. Confocal-immunofluorescence revealed that GRIP expression was particularly elevated in inhibitory Purkinje cell bodies (40%, $p<0.0001$). Subcellular immunogold analysis of GRIP at excitatory mossy fibre-granule cell (MF-GC) synapses, which are devoid of GluA2/3-AMPARs, showed no significant difference ($p>0.05$) between stargazers and controls. In the stargazer thalamus, the primarily GABAergic reticular thalamic nucleus showed a modest increase in GRIP; however, this was not statistically significant ($p>0.05$). PICK1 expression was unchanged in stargazer cerebellum and thalamus ($p>0.05$). **Conclusions:** Loss of synaptic GluA2/3-AMPARs does not alter GRIP expression at excitatory MF-GC synapses. Increased cerebellar GRIP levels may reflect compensatory changes at inhibitory neuron synapses. GRIP has been identified and proposed to facilitate GABA-receptor stabilisation. Understanding cell-specific changes in GRIP expression in stargazers may help identify common molecular mechanisms present in comorbid disorders like ataxia, epilepsy and autism.

POS-TUE-045

THE EFFECTS OF ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION ON PAIN THRESHOLD AND PAIN LEVEL: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Purpose: The primary aim is to check the literature for the effects of anodal transcranial direct current stimulation (a-tDCS) on sensory and pain threshold (STh, PTh) in healthy individuals and pain level (PL) in patients with chronic pain. The secondary aim is to find a-tDCS optimal parameters for its maximal analgesic effects. **Method:** Seven electronic databases were searched for the studies on the effects of a-tDCS when compared to sham and controls. Studies in which measured STh, PTh, and PL by numeric or visual analogue scale were included. Methodological quality was examined using PEDro and Down and Black (B&D) assessment tools. All studies examined the effects of a-tDCS intervention in different areas of brain related to pain processing, including primary motor and sensory cortex (M1, S1) and dorsolateral prefrontal cortex (DLPFC). **Results:** Data from 9 included studies revealed increase in PTh by stimulation of M1 (P= 0.003) in healthy individuals. The increase was not significant for S1 stimulation. Studies on patients with chronic pain showed significant decrease in PL in both M1 and DLPFC (P< 0.00001) stimulation. The result also indicates that, efficacy of a-tDCS depends on current density and duration of application in both healthy individuals and patients. **Conclusion:** A-tDCS is a non-invasive technique to increase PTh in healthy individuals and decrease PL in patients with chronic pain. Due to small sample size of included studies, interpretation of the results should be considered cautiously.

POS-TUE-046

SORTING RETROGRADE FROM LOCAL VESICULAR TRAFFICKING IN PRESYNAPTIC NERVE TERMINALS

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Both Cholera toxin (CTB) and Botulinum neurotoxin type-A heavy chain (HC-BoNT/A) are endocytosed at presynaptic nerve terminals. Whereas Botulinum neurotoxin is uptaken in recycled synaptic vesicles, Cholera toxin is retrogradely transported following its internalisation. The sorting mechanism(s) allowing these two critical trafficking pathways to co-exist in the crowded presynaptic environment are largely unknown. **Purpose:** To explore the underlying molecular mechanism that distinguishes local vesicular recycling from retrograde trafficking pathways utilizing fluorescently labelled HC-BoNT/A and CTB in the context of microfluidic chambers. **Methods:** We developed a time-lapse confocal microscopy assay using microfluid chambers and simultaneous application of HC-BoNT/A and CTB in the chamber containing nerve terminals to distinguish and quantify local recycling from retrograde trafficking. **Results:** We reveal that the presynaptic uptake of both HC-BoNT/A and CTB are both activity-dependent. In unstimulated conditions, considerably less CTB are retrogradely transported. A significant proportion of the retrograde CTB-positive carriers colocalized with the neurotrophin receptor TrkB (30.67±6.7%, n=5) and VAMP2 (27.08±9%, n=5). Most HC-BoNT/A localized to VAMP2-positive presynaptic terminals, but to our surprise some were also retrogradely transported together with Cholera toxin. However, upon depolarization, with high potassium, the frequency of CTB retrograde carriers was dramatically increased (2.233 ± 0.6769 fold, n=10, p=0.0042). We also noticed less HC-BoNT/A/CTB positive retrograde carriers. **Conclusion:**Our results suggest an activity-dependent sorting mechanism that maintains HC-BoNT/A in presynaptic nerve terminals while promoting CTB retrograde trafficking. These results suggest that active neurons have an increased ability to perform critical presynaptic vesicular sorting.

POS-TUE-047

CHARACTERIZATION OF THE ELECTROPHYSIOLOGICAL PROPERTIES OF NAV1.1 AND NAV1.2 EXPRESSED IN HEK293T CELL LINES

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Purpose: Nav 1.1 and 1.2 are two of the most commonly expressed forms of the voltage gated sodium channel in the mammalian CNS with 75% structural homology. Knowledge of the basic electrophysiological and kinetics of these channels will be important to understand the pathophysiology related to genetic disorders and the effects of sodium channel modulators on these subtypes. **Methods:** Sodium currents in HEK293T cells transiently expressing human Nav1.1 and Nav1.2 alpha subunits were recorded in the whole cell configuration. Activation, inactivation properties, equilibrium states as well as dynamic transitions between different states were examined with several protocols in a temperature controlled room (22-23°C). **Results:** Recordings were made from 61 cells. Steady- state activation was similar (n=5) in both channel subtypes. Steady state inactivation was measured with 50 ms, 500 ms and 10 second conditioning pulses to attempt to isolate inactivation processes with different time constants were found similar (Nav1.1, n=11; Nav 1.2, n=9). Differences were seen in dynamic situation. Nav 1.1 has 40% less channels (n=7) needed to recover in the potential range -70 to -100 mV and more Nav 1.2 channels entered inactivation with potentials changes from -100 to -60, -70, and -80 mV (n≥5). Nav 1.1 was less affected by use-dependent inactivation elicited by 40 Hz depolarization (n=6). Additionally, Nav 1.1 recovers from slow inactivation more completely (n=5). **Conclusion:** Nav 1.1 enters less and recovers more from inactivation at slower time scales. This may relate to its putative role as the major channel subtype in interneurons that might facilitate the high-frequency firing patterns seen in these cells. It will be of great interest to see the effect of slower inactivation modulating drug such as lacosamide in more specific studies.

POS-TUE-048

INCREASED α-SYNUCLEIN PHOSPHORYLATION IN MICE WITH TRANSIENT MPTP TOXICITY CORRELATES WITH INCREASED LEVELS OF POLO-LIKE KINASES

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Purpose: To use an animal model of Parkinson's disease (PD) to determine if α-synuclein is hyperphosphorylated due to increased levels of kinases following MPTP exposure. **Methods:** Following ethics approval, 36 C57/BL mice were administered MPTP-HCL (30mg/kg/day in saline) and 30 were administered saline control intraperitoneally for 5 consecutive days. Groups of 5-6 mice from MPTP and control groups were sacrificed at different time points following their last injection [1 day before (-1) as well as at 1, 3, 7, 14 or 28 days]. Frozen brain tissue was prepared for semi-quantitative western blotting of tyrosine hydroxylase (TH), α-synuclein (S129 phosphorylated and total) and related kinases [polo-like kinases (PLK1-3) and casein kinases (CKI-II)]. Multivariate analyses were used to identify changes and correlations over time. **Results:** During acute MPTP toxicity (-1 to 3 days), there was 1) a significant decrease in the levels of TH and total α-synuclein in the striatum, 2) a dramatic increase in the levels of phopshorylated α-synuclein in both nigra and striatum, and 3) a correlation between increased α-synuclein phopshorylation and increased levels of PLK-2 and PLK-3 in both the nigra and striatum. The levels of all proteins trended to return to normal during the recovery period (7 to 28 days). **Conclusions:** The acute reduction of TH and α-synuclein in the striatum of this MPTP model suggests acute synaptic toxicity with terminal retraction and cell body accumulation of proteins. During this time PLK-2/3 kinases increase and phosphorylate α-synuclein, suggesting that such a change is required for tissue recovery.

POS-TUE-049

IDENTIFICATION OF THE SENSORY NERVE ENDINGS THAT DETECT PAIN IN THE ESOPHAGUS AND STOMACH USING A NOVEL NEURONAL TRACING TECHNIQUE

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The sensory nerve endings that detect pain in the esophagus and stomach of mammals have not been identified, due to difficulties in identifying the particular classes of sensory nerve fibres that detect pain over the other sensory and motor fibres that innervate these organs. One of the major difficulties in studying "pain" fibres is the inaccessibility of identifying and preserving the extrinsic sensory neural pathways between the spinal cord and esophagus and stomach. It is clear, based on lesion studies, that the spinal afferent nerves, whose cell bodies lie in dorsal root ganglia are the sensory neurons which detect pain from these regions. To overcome these technical difficulties, we developed a novel in vitro preparation in which dorsal root ganglia were removed from mice, whilst retaining complete neural continuity with the stomach and esophagus in vitro. Purpose: To identify the morphology and target sites of innervation of the spinal afferent nerves that innervate in esophagus and stomach. Methods: Dorsal root ganglia (T7-T13) were removed with the entire stomach and esophagus preserved and cultured for 5 days. Primary antibodies to Calcitonin Gene Related Peptide (CGRP) was used to verify that labeled spinal nerve endings where primary afferents. Results: After 5 days in culture, CGRP immunoreactive fine varicose nerve endings were found to ramify within the fundus, corpus and antrum smooth muscle layers, and extensively within the myenteric ganglia, submucosa and mucosal barrier (N=5). To test that CGRP labeled sensory nerve endings were of spinal afferent origin, we also cultured DRGs (T7-T13) with stomach and esophagus attached, but made a complete lesion through the spinal nerves. In these preparations, no CGRP labeled fibres existed (N=4). The TRPV1 antibody was found to colocalize with all CGRP-positive spinal nerve endings (N=4). Conclusions: These findings show that TRPV1 positive (capsaicin-sensitive) spinal afferent nerve endings ramify within multiple sites of innervation in the esophagus and stomach as an extensive varicose arbor. The sensory nerve cell bodies of these spinal afferents lie primarily in the thoracic spinal cord (T7-T13).

POS-TUE-050

DEVELOPMENT OF A STANDARDIZED SYSTEM FOR PERCEPTUAL RIVALRY RESEARCH

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Purpose: Simultaneous viewing of different stimuli, one by each eye, induces perceptual alternations between each image every few seconds. Such binocular rivalry has been examined by scientists since the 1830s, and more recently has been a useful tool for dissociating neural correlates of perceptual awareness from those associated with visual presentation. Similar paradigms like continuous flash suppression have also been used in such studies. However, methods by which dichoptic images are presented have been mainly confined to specialist engineers, optical physicists and vision researchers. **Methods:** We describe the development of two prototype setups for standardized rivalry testing that can be utilized by non-specialized research staff. User-friendly operation was enabled by development of a software package that also resolves issues inherent with multi-monitor setups (e.g., video signal synchrony) and eliminating interference between concurrently run stimulus presentation and data collection functions. **Results:** Our system runs a specialized True3Di™/AOC™ monitor (for stimulus presentation) and a conventional monitor (for data acquisition) simultaneously via a single PC. These functions are synthesized with data analysis/management operated via a single user-friendly interface. This standardized design eliminates extensive programming and testing usually required for generating rivalry experimental protocols. **Conclusion:** The system we developed is standardized for use by investigators new to rivalry research and also in large-scale population-based studies (e.g., GWAS) where stimulus conditions and recording protocols need to be kept constant.

POS-TUE-051

NEURONS WITH ABSOLUTE DEPTH SENSITIVITY IN CAT VISUAL CORTEX

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Purpose: Neurons sensitive to relative depth have been described in different areas of the visual cortex, but there have been few reports of neurons with responses modulated by absolute depth. We studied the distribution of these cells in various visual cortical areas (V1, V2, V4A, Frontal visual area (FVA) in behaving cats. **Methods:** In Experiment 1 (3 cats) we searched for the areas with neuronal responses modulated by absolute distance. Responses to stimuli of the same angular size were recorded at near (20 cm) or far (3 m) distances, under monocular observation. Neurons were considered as depth modulated if their responses in these two conditions were different. In Experiment 2 (2 cats) we tested cells for their absolute distance selectivity versus spatial frequency selectivity. Gratings with different spatial frequencies were sequentially projected on a large screen and neuronal activity was recorded from many different distances. We were looking for the cells having maximal responses at the same distance independent of the spatial frequency. **Results:** In Exp 1, neurons selective to absolute depth (Wilcoxon T-test, $p<0.05$) were found in all tested areas except V2: 38 out of 130 cells recorded in V1, 0/34 in V2, 100/310 in V4A, 28/108 in FVA. In Exp. 2 in area V1 we found 26 distance-selective cells out of 116 (Sign test, $p<0.05$). **Conclusions:** Our results show that information regarding absolute distances to visual scene might be available at different stages of visual processing starting from the primary visual cortex.

POS-TUE-052

ALTERATIONS IN SYNAPSE-ASSOCIATED PROTEINS IN THE RETINAE OF A MOUSE MODEL OF TYPE 2 DIABETES

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Purpose: Diabetic Retinopathy is a major complication of Type 2 Diabetes and is the leading cause of blindness in working-aged adults. This study examined changes in the retinal membrane proteome of an established diabetes mouse model. **Methods:** Obese diabetic db/db mice were compared to a lean non-diabetic wild-type group, and to additional db/db mice receiving either metformin (300mg/kg*d), substance D (30mg/kg*d), or a combination of both from 8 weeks of age (n=10/group). The mice had been fed a high fat/high carbohydrate diet to ensure a uniform diabetic phenotype. At 10 weeks of age, the mice were culled and retinae collected and processed for membrane proteomic analysis. Candidate proteins were selected and studied by immunoblot and immunocytochemical analysis in a further cohort of WT and db/db mice (n=10/group). **Results:** Over 850 proteins were identified from the membrane-enriched fractions of the five groups. After filtration of the results, 77 proteins were found to be differentially abundant across all groups. Of these, 15 proteins were identified as being differentially abundant in the WT vs. non-treated db/db groups, but not when compared to the drug-treated db/db groups. The proteins altered by diabetes but not by drug treatment included Ctbp2/RIBEYE, Vglut1, and PMCA1, all of which are associated with ribbon synapses in retinal neurons. **Conclusion:** These data suggest that the abundances of proteins associated with ribbon synapses are susceptible in diabetic retinae. Further work is necessary to determine the mechanism causing these protein alterations.

POS-TUE-053

ENDOCANNABINOIDS MODULATE LIGHT SIGNALS IN THE RETINA

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Endocannabinoids and their receptors have been localized to all retinal cells. This system plays a role in plasticity and modulation of cell excitability throughout the brain, however the role of cannabinoids in retinal processing of light signals has not been investigated. **Purpose:** To investigate modulators of the endocannabinoid signaling pathway on light response properties of retinal ganglion cells (RGCs). **Methods:** Whole cell patch camp clamp recordings were taken from the RGCs in mouse retina in the whole mount preparation. Contrast sensitivity and area response function were measured by projecting visual stimuli on the photoreceptor layer. Recordings were obtained before and after the addition of a cannabinoid receptor agonist WIN55212-2 (10µm) and a cannabinoid receptor antagonist AM251 (5µm). Additionally, conductance measurements were calculated before and after the bath application of the agonist. **Results:** Addition of a cannabinoid agonist caused an all around dampening of the light response. Peak spike response was significantly reduced on average of 38.5% ($p<0.05$, n=24) and surround inhibition was reduced by 23% ($p<0.05$, n=22). Cannabinoid agonist also affected contrast sensitivity by reducing depolarization to a preferred stimulus by 43% ($p<0.05$, n=21) and hyperpolarization to a non-preferred stimulus by 59% ($p<0.05$, n=21). Addition of the cannabinoid receptor antagonist increased the peak response by 18% ($p<0.05$, n=13) and surround inhibition by 30% ($p<0.05$, n=13). **Conclusion:** These studies show that cannabinoids can modulate retinal processing. This is expressed as an all around dampening of both excitatory and inhibitory responses to light, furthermore a cannabinoid receptor antagonist has the inverse effect implying a tonic level of endogenous cannabinoid activity, thus suggesting an active role for cannabinoids in retinal processing.

POS-TUE-054

MICROGLIAL RESPONSE TO SUB-LETHAL RETINAL INJURY: A ROLE IN NEUROPROTECTION?

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Purpose: Microglia are the resident immune cells of the central nervous system and retina. While their pro-inflammatory role has been well documented, a number of recent studies have highlighted neuroprotective functions. This work details the microglial response to a sub-lethal retinal injury. **Methods**: A sub-lethal injury was induced in the mouse retina using a low energy laser (2RT laser, 0.065mJ). Retinal changes were assessed 1 hour to 7 days post-injury using immunohistochemistry and cell death was monitored using TUNEL (each n>5). Microglial response was characterised in a mouse in which microglia express green fluorescent protein (Cx3cr1GFP/+, n=9). PCR microarrays (n=3) were used to characterise retinal cytokine profile 5 hours after sub-lethal (0.065mJ) and lethal (0.13mJ) injury. **Results**: The sub-lethal injury produced no structural alteration, with no cell death or Müller cell gliosis evident. Despite this, retinal microglial response was rapid (1 hour), with photoreceptor-microglial interaction observed and extension of microglial processes into the sub-retinal space. Microglia showed no evidence of activation, with cell number, soma size and process morphology remaining unchanged. The sub-lethal injury resulted in up-regulation of genes involved in chemotaxis (Ccl2, Ccl7) and an increase in the neuroprotective cytokine, leukemia inhibitory factor (Lif). Increasing the severity of the retinal injury (0.13mJ) reduced the expression of possible neuroprotective agents (Ctf1, Il9). **Conclusion**: Sub-lethal retinal injury produced a rapid microglial response that was not a result of classical activation. The neuronal interaction and increased expression of neurotrophic agents suggests that microglia may aid in cell survival after mild retinal insult.

POS-TUE-055

α9-NICOTINIC ACETYLCHOLINE RECEPTORS CONTRIBUTE TO MAINTENANCE OF NERVE INJURY-INDUCED MECHANICAL HYPERALGESIA BUT NOT ALLODYNIA: A DUAL MECHANISM FOR α-CONOTOXINS?

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Purpose: Chronic pain is poorly managed pharmacologically. Conotoxins from marine cone snails are a source of potential analgesics. Vc1.1 is an α-conotoxin producing effective, sustained relief of mechanical allodynia and hyperalgesia in rodent models of neuropathic pain. Two molecular targets could mediate these actions. Vc1.1 potently and specifically inhibits nAChRs composed of α9 and α10 subunits. However, antagonism of the α9-nAChR has been suggested to be neither sufficient nor necessary for pain relief. Vc1.1 also inhibits N-type Ca2+ channel currents in a GABAB receptor-dependent manner, and *in vivo*, GABAB antagonists reverse acute Vc1.1 anti-allodynia. **Methods:** To determine whether activation of α9α10 nAChRs can contribute to chronic pain, several sciatic nerve injury models were tested in α9 nAChR-knockout (KO) and wild-type (WT) mice. **Results:** KO (n=6) mice develop mechanical allodynia (von Frey and incapacitance tests) that is indistinguishable from WTs (n=6), which persists for at least 3 weeks. Mechanical hyperalgesia (paw pressure test) also develops in the KO within 1 week (KO: 61±67% of pre-surgical response; WT: 45±55%, $p<0.001$, Bonferroni one-way ANOVA) but greater recovery is observed by the second week post surgery (KO: 89±55% of pre-surgical response; WT: 48±45%, $p<0.01$, Bonferroni one-way ANOVA). **Conclusion:** The results show that mechanical hyperalgesia is less persistent when the α9-nAChR is deleted but mechanical allodynia is unaffected. Perhaps, whilst the acute anti-allodynic effects of Vc1.1 do not involve the α9-nAChR, sustained relief of mechanical hyperalgesia may be achieved through α9-nAChR inhibition.

POS-TUE-056

DIFFERENTIAL SENSITIVITY OF NEONATAL AND ADULT RAT SENSORY NEURONS TO OMEGA-CONOTOXINS CVID AND CVIE

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Omega-conotoxins that selectively block N-type calcium channels have emerged as potential new therapeutics for the treatment of pain. We were interested in two omega-conotoxins, CVID and CVIE, which were found to have different effects in neonatal and adult rat dorsal root ganglion (DRG) neurons. Previous studies have shown that the reversibility of these omega-conotoxins is dependent on the differential expression of calcium channel subunits, which could potentially vary during development. **Purpose:** To measure the concentration-response and reversibility of omega-conotoxins CVIE and CVID in neonatal and adult DRG neurons. **Methods:** We performed whole-cell patch clamp recordings of VGCC currents in isolated DRG neurons from adult (>6 weeks) and neonatal (4-12 days) male rats. **Results:** Near maximal concentrations of CVID and CVIE inhibited the total ICa in all neurons (n=32) tested by 49 ± 4 %, with no significant difference between maximal inhibition by CVID and CVIE. In DRG neurons from adult rats, complete recovery was seen following washout of CVIE in all cells tested (n=9). Recovery from CVID was more variable, with no recovery in 4 cells, partial recovery in 1 and complete recovery in 6 out of 11 cells total. In DRG neurons from neonatal rats, recovery from CVIE block was tested in two cells, with complete recovery in one and partial recovery in the other. No recovery was seen following CVID block in 7 neurons, and partial recovery was seen in 1 out of a total of 8 cells tested. The recovery from CVID block was significantly different in adult and neonatal neurons ($P<0.0001$). No significant difference was seen in recovery from CVIE. **Conclusion:** Recovery from omega-conotoxin block in different in neonatal and rat DRG neurons.

POS-TUE-057

AFFECTIVE REGARD AND STIMULUS FREQUENCY CONTRIBUTE TO MODULATORY EFFECTS OF C-TACTILE FIBRE ACTIVATION ON MUSCLE PAIN

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Purpose: We recently showed that low-threshold unmyelinated mechanoreceptors, termed C-tactile (CT) fibres, mediate vibration- and brush-evoked allodynia during muscle pain. Conversely, in absence of background pain, CT-fibre activation has been shown to correlate with a diffuse sensation of pleasant touch. In this study, we investigated whether tactile modulation of pain, in particular the perceptual effect of CT-fibre activation, is influenced by affective attributes and frequency parameters. **Methods:** Psychophysical observations were made in 20 healthy subjects. High-precision overtly affective stimuli (velvet fabric and sandpaper) were applied to the skin of anterolateral leg in absence of background pain. Thenceforth, muscle pain was induced by infusing hypertonic saline (5%) into tibialis anterior muscle. Furthermore, high (200Hz) and low (20Hz) frequency vibrotactile stimuli were applied in order to test for frequency-dependent effects on pain modulation. These observations were repeated prior to and following conduction block of myelinated fibres (nerve compression). Moreover, vibration-evoked effects were tested following blockade of unmyelinated cutaneous fibres (low-dose anaesthesia). **Results:** In absence of muscle pain, subjects reliably linked velvet-stroking to pleasantness and sandpaper to unpleasantness (no pain). During muscle pain, this correlation predicted enhancement and attenuation of pain, i.e. allodynia and hypoalgesia, respectively. Furthermore, high-frequency vibration evoked allodynia, whereas low-frequency vibration produced hypoalgesia. These effects were significant, reproducible and persisted during blockade of myelinated fibres. Contrarily, blockade of unmyelinated cutaneous fibres abolished the vibration-evoked effects. **Conclusion:** These observations indicate that temporal coding need not be limited to discriminative aspects of tactile processing, but may contribute to affective attributes, which in turn predispose individual responses towards excitatory or inhibitory modulation of pain.

POS-TUE-058

KAINATE RESPONSES OF RAT INNER RETINAL NEURONS

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Kainate receptors mediate fast, excitatory synaptic transmission for a range of inner neurons in the mammalian retina. However, assigning kainate sensitive glutamate receptors to known retinal cell types and describing their functionality still remains a challenge. We used the cation channel probe 1-amino-4-guanidobutane agmatine (AGB) to investigated the sensitivity of neurochemically identified cell populations to kainate within the intact rat retina. OFF cone bipolar cells were the most kainate sensitive inner retinal neuron population with a K_m of ~5μM. Co-localization of AGB and a marker for Type 2 OFF bipolar cells confirmed kainate sensitivity in this subpopulation. Most amacrine and ganglion cells responded to kainate in a concentration dependent manner. Cholinergic amacrine cells were highly kainate responsive whilst GABAergic amacrine cells were the more kainate sensitive then glycinergic amacrine cells. For ganglion cells, glutamatergic cells were the most sensitive followed by glutamatergic/weakly GABAergic ganglion cells. These findings further contribute deciphering signalling pathways and neuronal networks in complex multi-cellular tissues.

POS-TUE-059

MORPHOLOGICAL CHANGES OCCUR IN SOME GANGLION CELLS IN RETINITIS PIGMENTOSA

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Purpose: Retinitis pigmentosa (RP) refers to a family of inherited photoreceptor degenerations resulting in blindness. Our research focuses on characterising the inner retina following complete photoreceptor death. We have previously used a novel transgenic mouse, *rd1*-FTL, to describe regions of the retina which show both an increase in c-*fos* expression and a glial dysfunction at late stages of degeneration. Here, we have investigated whether any morphological change in ganglion cells occurs in these regions. **Methods:** We used triple mutant transgenic mice, *rd1*-FTL-Thy1, with a mutation in the β subunit of phosphodiesterase 6 leading to RP, an axon-targeted β-galactosidase reporter system which is regulated by the c-*fos* gene and a fluorescent protein that labels a subset of ganglion cells. P90 to P365 *rd1*-FTL-Thy1 and control mice were prepared as wholemounts and processed using immunohistochemistry. **Results:** Ganglion cells (cells=1065, n=28) were classified based on soma area, dendritic field size and branching of dendrites. The dendritic fields of some ganglion cells (cells=605, n=28) were further analysed for their length, area and quantity of branching points. Interestingly, there was a decrease in size and complexity of A type ganglion cells in the degenerated retina from P90. In contrast, the smaller cell types, B and C, remained unchanged. However, at P330, morphology changes of these smaller cells types were observed in regions that also expressed c-*fos*. This indicates that the integrity of neighbouring neurons and glia may impact surviving ganglion cells at later stages of degeneration. **Conclusion:** We propose these changes in ganglion cell morphology will most likely impact the function of individual cells as well as the retinal circuitry in the degenerated retina.

POS-TUE-060

STRENGTH OF THE RUBBER HAND ILLUSION AFFECTS SENSORIMOTOR PROCESSES

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When tactile stimulation is applied simultaneously to a (seen) fake hand and the (unseen) real hand, the felt touch can be misperceived as being located on the fake hand. This integration between the visual and tactile sensory inputs is also associated with drift in the perceived position of the real hand towards the fake hand and a heightened sense of ownership for the fake hand. This paradigm, the rubber hand illusion, has been employed as an experimental tool in research that aims to better understand multisensory integration and the perception of body ownership. In the present study, we vary the degree of spatial discrepancy between the real and fake hands when the illusion is induced in a sample of healthy participants (n=17). Consistent with previous studies, the perceptual effects of the illusion are reduced in strength when there is greater spatial discrepancy between the real and fake hands. In addition, we discover that reaching movements performed following illusion induction are similarly affected by the position of the fake hand. These results suggest that the neural representation(s) of hand position used to execute reaching movements are sensitive to the degree of conflict between the sensory inputs used to estimate hand position.

POS-TUE-061

RETINAL OXYGEN SATURATION AS A FUNCTION OF ARTERIAL AND VENOUS SIZE

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Purpose: We employ a novel whole-image-based analysis technique to investigate the relationship between oxygen saturation and blood vessel width in superior and inferior retinal hemifields. **Methods:** Ten images were acquired from a randomly selected eye of 17 healthy participants (age 22-38) using the Oxymap T1 retinal oximeter. All pixels segmented by the vessel detection algorithm were analysed by plotting frequency histograms for vessel diameter bins of 10µm (70-170µm). The average histogram for 10 images was modelled using two Gaussian functions returning peak oxygen saturation at each vessel width. Mean (±SEM) high and low oxygen saturation at each vessel width was calculated. Data were also analysed after segregating pixels into upper and lower hemifields demarcated by the centre of the optic nerve. Two-way ANOVA was used to compare oxygen saturation in arteries and veins of all diameters. **Results:** Oxygen saturation in arterioles was highest in large vessels, and stable for vessels greater than 100µm (95±1%). For vessels between 120 and 60µm, oxygen saturation changed at -2.6% for each 10µm reduction in vessel width. The highest oxygen saturation was found for veins (75±1% at 70µm) less than 100µm. For larger veins, oxygen saturation changed at -2.4% for each 10µm increase in vessel width. Oxygen saturation was higher in the upper hemisphere for arteries (mean difference +2±1%, $p<0.01$) and veins (+2±1%, $p<0.01$). This difference was more pronounced in large compared with small veins (large 6±2%, small 1±4%). **Conclusions:** Retinal vessel oxygen saturation can be modelled as a function of vessel width. Oxygen saturation was lower overall in the lower retinal hemifield.

POS-TUE-062

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

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Purpose: We previously reported that some neurons in the intercalated (koniocellular, KC) layers of the lateral geniculate nucleus (LGN) show high variability in maintained discharge rate. Discharge rate is inversely correlated to low frequency power in the electroencephalogram (EEG) in the primary visual cortex (V1) [1]. Our purpose here is to find the source of this variability, specifically its time-relation to low frequency EEG. **Methods:** Extracellular spike activity of LGN neurons (n=107) and local field potential from primary visual cortex (V1) were recorded in sufentanil-anaesthetised marmosets (Callithrix jacchus, n=12). The visual stimulus was a uniform grey field ~15 degrees square at close to 50 Cd / m^2. Granger causality analysis was performed on LGN neuron maintained discharge rate and V1 delta frequency EEG strength using a 9 second moving window with 0.3 second steps. We used a model order of 5 as calculated by Akaike information criterion. Data are expressed as mean±SD. **Results:** As reported[1] KC neurons show high variance in maintained discharge rate variance (36.83±46.72, n=37) compared to Parvocellular (9.13±9.84, n=45) and Magnocellular (12.55±15.19, n=25) neurons. Granger causality analysis of 7 epoch from 2 KC neurons showed that on average the power in delta EEG is better in predicting firing rate in LGN (1.9±0.82) than LGN firing rate is in predicting delta EEG power (0.38±0.2). **Conclusion:** These results indicate that decreases in delta frequency oscillation strength in the primary visual cortex cause increased activity in the KC layers of the LGN. [1] Cheong S.K. et. al., (2011) *PNAS* **35**, 14659-14663.

POS-TUE-063

TOPOGRAPHY OF PREFRONTAL CONNECTIONS TO THE CLAUSTRUM OF THE COMMON MARMOSET *(CALLITHRIX JACCHUS)*

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Purpose: Although its existence has been known for over 150 years, the function of the mammalian claustrum remains obscure. We have attempted to map the connectivity between the prefrontal cortex and the claustrum in order to clarify how the claustrum may function in circuits involving executive or "top-down" cortical input. **Methods:** Neuroanatomical tracer injections were placed in prefrontal areas of 13 marmosets, under Alfaxan (10 mg/kg) anaesthesia. The injections targeted various subdivisions of Brodmann's areas (BA) 8, 9, 10, and 32. Following survival times of approximately 2 weeks, the number and spatial distribution of retrogradely labelled neurons were assessed following using fluorescence and light microscopy, and computer graphic reconstructions. **Results:** All targeted prefrontal areas yielded dense claustrum connections. The most comprehensive injection site coverage was for the BA8 complex, which produced a clear topographic segregation of claustrum connections: labelled cells projecting to the medial subdivision (area 8b) were restricted to the rostral claustrum, while cells projecting to the ventral subdivision (8aV) were clustered in the caudal claustrum. Cells labelled following area 9 injections were also more numerous rostrally. Current data confirm a previously described difference between the claustrum projections to ventromedial and dorsal BA10 (Burman et al. (2011) Eur J Neurosci 34(2):303). **Conclusions:**: The primate claustrum has a dense and specific pattern of prefrontal connections, which is only beginning to be understood. However, our data suggest that there may be functional segregation within the claustrum between connections involving sensory information (e.g. area 8aV), and those which subserve functions related to monitoring or changing of internal states (e.g. BA 10 and 8b).

POS-TUE-064

HALTING THE PROGRESSION OF NOISE-INDUCED HEARING LOSS WITH GENE THERAPY

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Background: Progressive hearing loss is often ignored until there is significant loss of cochlear hair cells (HCs) and spiral ganglion neurons (SGNs). It usually begins as a mild high-frequency threshold shift which worsens and also spreads to the lower frequencies. Our research indicates that gene therapy is effective for long-term preservation of SGNs when administered shortly after ototoxic hearing loss, but has the potential to protect residual HCs and SGNs after the onset of progressive hearing loss and even to restore hearing. **Purpose:** To establish the efficacy of gene therapy in a progressive noise-induced hearing loss model. **Methods:** Guinea pigs were exposed to 130 dB closed-field noise for 2 hours (10-14 kHz notch) under anaesthesia (n=15). Hearing was monitored by auditory brain-stem response (ABR). Gene therapy was by injection of adenoviral vectors expressing GFP into the scala media of the cochlea. **Results:** Noise exposure resulted in a 40-50 dB high-frequency threshold shift that was apparent at 2 weeks post-deafening ($p<0.05$ compared to pre-deafening). At this time point all animals had microscopically normal inner and outer HCs and supporting cells which were transduced by gene therapy vectors, particularly in the basal turn high-frequency region of the cochlea. At 5 weeks post-deafening, there was no further significant change in hearing thresholds. However, 83% of animals had loss of inner and outer HCs and supporting cells in the lower basal turn, demonstrating a progression of the lesion. **Conclusion:** Gene therapy in the scala media of the cochlea is well suited to a progressive hearing loss model as it targets the high frequency region of the cochlea and requires intact HCs and supporting cells for transduction. If gene therapy is administered to the cochlea before SNHL becomes too severe, gene therapy has the potential to protect HCs and SGNs from degeneration.

POS-TUE-065

HCN CURRENT: A MECHANISM ADJUSTING THE MEMBRANE PROPERTIES, EXCITABILITY, AND ACTIVITY PATTERN OF THE GIANT CELLS IN THE RAT DORSAL COCHLEAR NUCLEUS

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Purpose: Giant cells of the cochlear nucleus are thought to integrate multimodal sensory inputs and participate in monaural sound source localisation. Our aim was to explore the significance of a hyperpolarization-activated current in determining the activity of giant neurones prepared from 10-14-day-old rats (n=59). **Methods:** Patch-clamp experiments were performed using a brain slice preparation. **Results:** When subjected to hyperpolarizing stimuli, giant cells produced a ZD7288-sensitive inward current with a reversal potential and half-activation voltage of -36 and -88 mV, respectively. Consequently, the current was identified as the hyperpolarization-activated non-specific cationic current (I_h). At the resting membrane potential, 3.5% of the maximum I_h conductance was available. Inhibition of I_h hyperpolarized the membrane by 6 mV and impeded spontaneous firing. The frequencies of spontaneous inhibitory and excitatory postsynaptic currents reaching the giant cell bodies were reduced but no significant change was observed when evoked postsynaptic currents were recorded. Giant cells are affected by biphasic postsynaptic currents consisting of an excitatory and a subsequent inhibitory component. Inhibition of I_h reduced the frequency of these biphasic events by 65% and increased the decay time constants of the inhibitory component. **Conclusion:** I_h adjusts the resting membrane potential, contributes to spontaneous action potential firing, and may participate in the dendritic integration of the synaptic inputs of the giant neurones. This current may be especially important during the postnatal maturation of the auditory system.

POS-TUE-066

NOVEL ω-CONOTOXINS POTENTLY INHIBIT N-TYPE VOLTAGE–GATED CALCIUM CHANNELS IN SENSORY NEURONS AND PARTIALLY REVERSE PAIN BEHAVIOR AFTER SYSTEMIC DOSING

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ω-Conotoxins, selective inhibitors of N-type voltage–gated calcium channels (VGCCs) are a new class of pain therapeutics. They are usually administered intrathecally. Intravenous injection of ω-conotoxin CVID produced antihyperalgesic effects with less serious side effects than other ω-conotoxins. Purpose: to measure the effects of ω-conotoxins CVID, CVIE and CVIF, and their analogues CVIE(R10K) and CVIF(R10K) in isolated DRG neurons and a mouse model of inflammatory pain. Methods: We performed whole-cell patch clamp recordings of VGCCs from isolated mouse DRG neurons to investigate potency and reversibility of the different ω-conotoxins and assessed effects of systemic administration of the ω-conotoxins in a CFA pain model in mice by measuring hind paw weight bearing and von Frey thresholds before and 1, 2, 4, and 6 h after s.c. administration of a range of ω-conotoxin doses. Results: In DRG, CVIE (IC50=6.5 nM) was more potent than the other ω-conotoxins. Both (R10K) analogues were completely reversible, CVIF was partially reversible (23±8%), and CVID and CVIE were irreversible. All peptides produced significant partial reversal of inflammatory pain at a dose of 2 mg/kg (n=8, P< 0.05 Dunnett's post-hoc one-way ANOVA) with a peak effect at 2 h post-injection. CVIE was significantly more potent than CVIF (at 2.0 mg/kg, P < 0.01 Bonferroni two factor ANOVA) and CVIF(R10K) (at 0.2 mg/kg, P < 0.05) but not CVID or CVIE(R10K). Conclusion: Systemically administered ω-conotoxins were found to alleviate inflammatory pain without side effects. There was a discrepancy in the potencies in vivo and in vitro, possibly due to differences in bioavailability.

POS-TUE-067

DEVELOPMENT OF A NOVEL NEURONAL TRACING TECHNIQUE TO REVEAL THE PROJECTIONS OF SPINAL AFFERENTS TO THE COLORECTUM

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In the gastrointestinal tract, it has been presumed that the sensory division of the sacral spinal cord provides an innervation primarily, if not solely, to the terminal large bowel and rectum. However, this has not been possible to confirm directly, due to the absence of a selective neuronal tracing technique that labels sensory axons only, while excluding labeling from motor fibres. To overcome this, we developed a novel in vitro preparation in which dorsal root ganglia were removed from mice, whilst retaining complete neural continuity with the large intestine in vitro. Purpose: To identify exactly how far spinal afferent nerves innervate in rostral-caudal direction along the large bowel; and identify their specific target sites. Methods: Lesions were made to the lumbar splanchic and hypogastric nerves, so that only rectal/pelvic nerves were retained between DRG and large intestine. Cholera toxin B was then injected into DRGs at L6-S4. Results: After 7 days, anterogradely labeled axons were found to ramify up to 18mm oral and 9mm anal along the colorectum from their point of entry into the colorectum (N=11). To further test this, we injected oregan green dextran into the proximal colon 5mm from the cecum. Retrogradely labeled DRG cell bodies were identified in S1-S4 (N=2). Anterogradely labeled (TRPV1 positive) spinal nerve endings were identified in the circular muscle, myenteric ganglia, submucosal ganglia, blood vessels, mucosa and occasionally longitudinal muscle layer (N=4). Conclusions: These findings show that rectal/pelvic spinal afferents, which are the primary pain fibres to the colorectum, and whose sensory nerve cell bodies lie in the sacral spinal cord (S1-S4), send direct axonal projections as far as the proximal colon.

POS-TUE-068

THE EFFECTS OF THE SEROTONERGIC DRUGS CITALOPRAM AND BUSPIRONE ON PERCEPTUAL RIVALRY

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Purpose: When sensory input is truly ambiguous, conscious perception tends to switch between the two mutually exclusive interpretations in a phenomenon known as perceptual rivalry. Previous research has suggested that the timing of these switches can be altered by a range of serotonergic drugs that either selectively or non-selectively activate the 5-HT_{1A} receptor. We aimed to investigate whether this change in perceptual rivalry switch rate was due to global levels of serotonin in the brain, or to specific activation of the 5-HT_{1A} receptor. **Methods:** We used two serotonergic drugs in healthy participants (n=12): citalopram to increase global levels of serotonin, and buspirone to activate 5-HT_{1A} receptors. Perceptual testing included binocular rivalry and auditory stream segregation, and participants were asked to indicate when their conscious awareness of the stimulus changed between the two possible perceptual states. **Results:** While several individual participants showed changes in switch rate across conditions, the direction of change was not consistent across participants. Therefore, there were no significant differences in switch rate between citalopram, buspirone, and placebo conditions in visual and auditory paradigms. **Conclusion:** Unlike previous studies, we did not find evidence that changes in serotonin affect the rate of switch in perceptual rivalry. It is possible that participants' general arousal levels may have been affected by other methodological factors, such as refraining from caffeine and the extended waiting time required for drugs to reach peak plasma levels, and that these factors interacted with the affects of the drugs on rivalry switch rate. Individual differences in reactions to the drugs may also play a role.

POS-TUE-069

IGNORING THE ELEPHANT IN THE ROOM: WHICH ATTENTION CUES AID IN DISTRACTER SUPPRESSION

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Sustaining visual attention is just as important as deploying attention for effective task performance. However, remaining focused on a task is often difficult, especially when faced with distracters. **PURPOSE:** In this study, we assessed the effectiveness of location and feature cues in the presence and absence of a highly salient distracter. **METHODS**: Attention was cued to one of four locations or one of two features of coherently moving dots (target), while at the other locations sets of randomly moving dots (noise) were presented. When location of the target was cued, participants (n=7) reported the direction of the coherently moving dots (upward or downward) at the cued location. When features of the coherently moving dots (i.e. a combination of direction of motion and colour) were cued, participants indicated which of four locations displayed the cued feature. In half the trials, a 100% coherently moving distracter was also presented at one of the non-target locations. This distracter had features that were either similar or dissimilar to the target. **RESULTS**: It was found that the presence and absence of a salient distracter did not affect performance when location was cued (Paired t-test, $P>0.05$) but when feature was cued, the type of movement in the distracter influenced performance (Repeated Measures ANOVA, $P<0.05$). Distracters that were similar to the target were harder to ignore than those that were different. **CONCLUSION**: These results are consistent with the theory that location based attention is more effective than feature based attention as it is relatively more resistant to the detrimental effects induced by a distracter.

POS-TUE-070

CCL2/CX3CR1 KNOCK-OUT MICE HAVE INNER RETINAL DYSFUNCTION BUT ARE NOT AN ACCELERATED MODEL OF AGE RELATED MACULAR DEGENERATION

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Purpose: The chemokine, Ccl2, and the fractalkine receptor, Cx3cr1, have both been implicated in the pathogenesis of age related macular degeneration (AMD), with mice lacking both genes exhibiting features of AMD by 3 months of age. However, recent reports indicate that this ascribed phenotype is due to the presence of a retinal degeneration mutation ($crb1^{rd8/rd8}$, rd8) on the background strain. Our aim was to characterise the retinal effects of lack of Ccl2 and Cx3cr1 ($Ccl2^{-/-}$/ $Cx3cr1^{EGFP/EGFP}$, CDKO-mice), in mice without the rd8 mutation. **Methods:** Nine month old, CDKO- (n=14) and wildtype C57blk6J-mice (n=18) were investigated for retinal fundus appearance and histology. The function of the rod and cone pathways was assessed using the electroretinogram (ERG). **Results:** The CDKO-mice did not develop lesions in the retinal fundus, and the ultrastructure of Bruch's membrane and the RPE were similar to that of C57blk6J-mice. From the ERG, there was no change in the amplitude of the rod photoreceptor response, or in the rod or cone post-photoreceptor b-wave. However, the rod and cone ERG oscillatory potentials were significantly reduced in the CDKO-animals, a phenotype apparent in $Cx3cr1^{EGFP/EGFP}$- but not $Ccl2^{-/-}$-founder lines. This correlated with aberrant amacrine cell morphology in the CDKO-mice. In addition, the Müller cells were gliotic and microglial morphology subtly altered, indicative of retinal stress. **Conclusion:** These results suggest that in the absence of the rd8 mutation, the CDKO-mouse has a mild inner retinal phenotype characterised by altered amacrine cell function, but that it is not an accelerated model of AMD.

POS-TUE-071

ORIGIN OF CONTRAST INVARIANCE OF CELLS IN THE CAT PRIMARY VISUAL CORTEX: CORTICAL OR SUBCORTICAL?

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Purpose: The invariant orientation sensitivity of striate cortical neurones to stimulus contrasts is often pointed out as a failure of Hubel & Wiesel's model of excitatory convergence (ECM). However, several studies have claimed that ECM could explain contrast invariance by taking into account the gain-contrast relationship and increase in noise fluctuations for low contrast stimuli of membrane potential responses. The source of such intriguing increase of variance at low contrast is unknown. We tested if contrast invariance is already present among lateral geniculate neurones (LGN), since responses of many LGN cells exhibit a bias for stimulus orientation. **Methods:** We recorded the extracellular responses from 15 LGN cells (in two anesthetized and paralysed cats) for thin, long, slowly moving bars of varying contrasts and orientation. Orientation biases were quantified as orientation sensitivity index (OSI) and circular variance (CV) at low and high contrasts for each LGN cell. **Results:** We found that the mean orientation sensitivity was not significantly different ($p>0.1$, Wilcoxon signed-rank test) between low (mean±SEM; OSI: 0.50±0.04; CV: 0.86±0.02) and high (mean OSI: 0.42±0.04; CV: 0.89±0.02) contrasts. We also found that the variance of spike rate at orthogonal orientations increased at low contrast ($n=9$; $p<0.05$, Wilcoxon test), while no significant change was observed at the optimal orientation ($n=9$; $p>0.05$, Wilcoxon test). **Conclusion:** The contrast invariance seen in striate cell membrane potential responses appears to be present in the output of LGN neurons. This adds further support to the idea that responses of single LGN cells could predict the properties of striate neurones, such as orientation preference & contrast invariance.

POS-TUE-072

QUANTIFICATION OF AMACRINE CELL POPULATIONS IN CENTRAL RETINA OF THE MARMOSET *(CALLITHRIX JACCHUS)*

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Purpose: There are at least 30 amacrine cell types in the retina but the specific functional role is only known for three to four types. Nearly all amacrine cells use either GABA or glycine as neurotransmitters but many also contain other neuroactive substances such as calcium binding proteins, dopamine, acetylcholine and nitric oxide. Our purpose was to characterize the density and distribution of these multiple amacrine subtypes in central (foveal) primate retina. **Method:** Antibodies against glutamic acid decarboxylase 65 (GAD-6) to label GABAergic amacrine cells, glycine transporter 1 (GlyT1) to label glycinergic amacrine cells, cholinergic acetyltransferase (ChAT), calbindin (CaBP), secretagogin (SCGN) and nitric oxide synthase (NOS) were applied to whole mounts and/or vertical sections of marmoset retina (n=3). Sections were analysed using confocal laser scanning microscopy and differential interference contrast (DIC) imaging. **Results:** In the inner nuclear layer (INL) at 1 mm from fovea centre, the peak density of 36000 amacrine cells/mm^2 (measured using DIC optics) is close to the sum of GABAergic (17707 cells/mm^2) and glycinergic (19776 cells/mm^2) amacrine cells. In the ganglion cell layer (GCL) no glycinergic cells are present and the density of GABAergic (GAD-6 positive) amacrine cells is 3073 cells/mm^2. In the INL, many GABAergic amacrine cells are also positive for Chat (19% of GABAergic amacrine cells), NOS (3%) or calbindin (12%). In the ganglion cell layer the corresponding percentages are 88% (Chat) and 5% (NOS). Secretagogin-positive cells made up 1% of the amacrine cells in the INL and 9% in the GCL. **Conclusion:** Central primate retina contains diverse neurochemical classes of amacrine cells.

POS-TUE-073

MOUSE RETINAL FUNCTION IS ALTERED BY SILDENAFIL (VIAGRA) ADMINISTRATION

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Purpose: To investigate the effects of the phosphodiesterase inhibitor, sildenafil (Viagra, Pfizer) on electroretinographic (ERG) responses in wild-type mice and heterozygous carriers of the rd1 mutation. Methods: Male C57 BL/6J mice and heterozygous wt/rd1 mice, aged ~55-90 days, received an intraperitoneal injection of sildenafil (Sigma) at either 20x or 200x the recommended human dose per bodyweight. Dark-adapted ERG responses were recorded through dilated pupils before and either 1 hour or 2 days post injection using a rodent ERG set-up. Results: In normal mice, at 1 hour post injection, a-wave responses were comparable to the pre-injection measures at 20x, but were severely reduced at 200x. B-wave amplitude was diminished at low-to-moderate stimulus intensities, relative to pre-injection measures. Suppression of b-waves was more evident for 200x than 20x. For 20x b-wave was undetectable below -1.5 log cd.s/m2, and for 200x b-wave was undetectable below -0.3 log cd.s/m2. At higher intensities above those thresholds, responses rose sharply to normal levels. At 2 days post injection a- and b-wave amplitudes were slightly reduced at high intensities. At 1 hour post injection in heterozygous wt/rd1 mice, the overall reduction in the b-wave was evident at low-to-moderate light levels but at higher light levels showed a dramatically increased response above those found pre-injection. Conclusion: Sildenafil has qualitatively different effects on dark-adapted ERGs at 1 hour and 2 days post-injection in normal mice. Differential responses in heterozygous carriers suggest influence of the rd1 mutation.

POS-TUE-074

ABSOLUTE STRENGTH OF AN ATTENDED STIMULUS IS ENCODED VIA COMPETITIVE SELECTION IN A VISUAL NEURON

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A lion focused on a single zebra within a panicked herd, or a dragonfly capturing flies amidst swarms of prey and conspecifics, are each selecting a single object amongst distracting stimuli. Although many animals can accomplish this attentional task, little is known about the neuronal mechanisms underlying the competitive selection of one moving feature from alternatives (against complex, textured backgrounds). Diverse evidence, from functional imaging and physiology to psychophysics, highlights the importance of 'competitive selection' in attention for vertebrates, artificial intelligence and even fruitflies, yet direct neural correlates are scarce from all animal groups[1]. Here we demonstrate responses from an identified dragonfly visual neuron, the centrifugal small target motion detector (CSTMD1), that perfectly match a model for competitive selection within the limits of neuronal variability (r2=0.83). Responses to individual targets presented at different locations of the receptive field differ in both magnitude and time course. However, responses to two simultaneous targets exclusively track those for one target alone. Successive repetition of stimulus pairs over variable sizes, separation and contrasts all elicit responses equivalent to single targets, regardless of whether the winner is the stronger stimulus if presented alone. We compare these responses to various models of neural interactions (e.g. summation, averaging) and show that the best match is to a model for competitive selection (Dunnett's multiple comparison $P<0.001$, $n=72$). CSTMD1 perfectly preserves the strength of the attended stimuli even in the presence of a salient distracter and this absolute encoding of stimuli simplifies downstream control systems for prey pursuit amidst swarms of distracters, versus scenarios where the prey is the sole salient feature within a scene. CSTMD1 is amenable to electrophysiological recordings, thus providing neuroscientists with a new model system of the competitive selection processes underlying visual attention. [1] Knudsen, E.I. (2007). Fundamental components of attention. Annu. Rev. Neurosci. 30:57-78.

POS-TUE-075

ULTRASTRUCTURAL CHANGES IN THE DEAFENED GUINEA PIG COCHLEA FOLLOWING TREATMENT WITH NEUROTROPHINS AND ELECTRICAL STIMULATION

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Purpose: Spiral ganglion neurons (SGNs) in the deafened cochlea undergo continual degeneration ultimately leading to 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. We have examined the effects of deafness duration on SGNs degeneration and the effects of delivery of brain derived neurotrophic factor (BDNF) with or without chronic ES. Methods: Adult guinea pigs (n=19) were deafened with ototoxic aminoglycosides and two weeks later implanted with an electrode array containing a cannula for NT delivery. A clinical device was used to deliver chronic ES over a four week treatment period. In a separate cohort, guinea pigs were deafened for 4 or 12 weeks to examine the effects of deafness duration. Cochleae were collected and prepared for examination on a transmission electron microscope. Results: SGN degeneration was characterised by retraction of the peripheral processes, shrinkage of the cell soma and ultimately cell death that was progressive over time. NT treatment reduced 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 ($p<0.0005$). 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

ORGANIZATION OF AREA MT IN MARMOSETS WITH EARLY V1 LESIONS

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Purpose: The developing brain is believed to be more malleable and more resistant to damages. In the primate visual system, however, the consequences of early-life brain lesions have not been examined in detail. After lesioning of the primary visual cortex (V1) in adulthood, limited form of plastic reorganization has been reported in extrastriate area MT. In this study we aimed to investigate if greater recovery can be found in animals lesioned early in life. Methods: V1 of 5 marmosets were partially and unilaterally ablated at 2 and 6 postnatal week. After long-term recovery, the response characteristics of MT neurons were studied. For each animal, the scotoma was first delineated by mapping V1 receptive fields along the perimeter of the lesion. MT receptive fields were then mapped and characterized. Results: We quantified 261 receptive fields in 5 animals. In all animals, responses were robust and consistent across the entire MT. As the depth of electrode increased, receptive fields moved in a pattern that was consistent to the canonical visuotopic organization of MT, moving across the scotoma without interruption. Surprisingly, direction selectivity of receptive fields inside the scotoma, but not those outside, was greatly reduced. 76.6% of the receptive fields outside the scotoma were direction selective, but only 22.8% of those inside satisfied the same criterion. The distributions of circular variance were statistically different (Kolmogorov-Smirnov $D=0.5269$, $p<0.001$). Conclusion: We found no evidence of disrupted or disorganized visuotopy, in contrast to what was found in animals lesioned in adulthood. Neurons in the deafferented region of MT, however, were much less selective to the direction of motion.

POS-TUE-077

CONTRAST-DEPENDENT PHASE SENSITIVITY OF COMPLEX CELLS IN MOUSE PRIMARY VISUAL CORTEX

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Purpose: The mammalian primary visual cortex (V1) consists of two classes of neurons: simple and complex cells. When presented with a moving sine-wave grating, phase-sensitive simple cells produce responses that oscillate in phase with the grating, whereas complex cells exhibit largely unmodulated responses. However, the phase-sensitivity of V1 neurons is not a fixed property. We have demonstrated in cats that the responses of a subset of complex cells become more phase-sensitive when the stimulus contrast is reduced. This phenomenon is consistent with a hierarchical model in which complex cells receive multiple simple cell inputs. We hypothesise that these simple cells exhibit different contrast response functions and that as contrast is reduced, inputs from simple cells with high contrast threshold 'drop off', resulting in more phase-sensitive responses produced by the remaining simple cell inputs. To test this hypothesis we examined the phase-sensitivity of the complex cells in mouse V1. **Methods:** We recorded extracellular spiking responses from 56 cells in mouse V1 while presenting drifting luminance modulated sine-wave gratings at 12 different contrasts. The phase-sensitivity of complex cell responses was then compared between different stimulus contrasts. **Results:** We found that 18% of recorded complex cells showed significant negative correlations between contrast and phase-sensitivity (t-test, $P < 0.001$). **Conclusion:** Similar to our observations in cats, reducing stimulus contrast increases phase-sensitivity of a subset of complex cells in mouse V1. This adds to evidence suggesting that the mouse is a suitable model for investigating cortical visual processing.

POS-TUE-078

SPATIAL DISTRIBUTION OF CORRELATED NEURONAL ACTIVITY IN MARMOSET LATERAL GENICULATE NUCLEUS

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Purpose: Visual signals relaying through the dorsal lateral geniculate nucleus (LGN) are modified by extra-retinal inputs. These inputs can induce correlated activity in relay cells and thus reduce the fidelity of visual signal transmission; hence they are called noise correlations. Here we asked whether noise correlations are spatially restricted or widespread in the LGN of marmoset monkeys (Callithrix jacchus). **Methods:** Extracellular recordings of neuronal activity were made in the LGN of Sufentanil-anaesthetised adult marmosets ($n = 2$). We used a NeuroNexus 32 channel probe comprising two shanks separated by 0.5 mm; each shank has 16 recording points separated by 0.05 mm. Isolated single-cell activity from 19, 25, and 25 cells at three sites was recorded and noise correlation calculated. Recording sites in LGN were verified anatomically. **Results:** Strength of noise correlation fell with distance between recording points in the range 0.1 - 1 mm (Pearson's correlation coefficients -0.22, -0.28, -0.29, $n > 100$, $p < 0.05$ for each site). Noise correlations between sites driven by the same (ipsi- or contralateral) eye were stronger (mean 0.063) than noise correlations between sites driven by different eyes (mean 0.031, $P < 0.05$, Wilcoxon rank-sum test). **Conclusions:** Noise correlations are propagated by at least 1 mm within marmoset LGN. Persistence (although weaker) of correlations at sites driven by different eyes supports extra-retinal source.

POS-TUE-079

TDP-43 AND STRESS GRANULE FORMATION IN RESPONSE TO ATP DEPLETION

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Mutations in the gene encoding Transactivation response DNA binding protein-43 (TDP-43) are commonly evident in the pathogenesis of two terminal neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTD). Structurally, TDP-43 belongs to a family of RNA-binding proteins known as heterogeneous nuclear ribonucleoproteins (hnRNPs). Conventionally, TDP-43 resides in the nucleus where it exerts its biological role in transcription, pre mRNA splicing, transport and regulating the stability of mRNA. However, in pathological tissue TDP-43 is relocalised to the cytoplasm where it is hyperphosphorylated, ubiquitinated, and cleaved resulting in lower molecular weight C-terminal fragments of 35 and 25 kDa. Recent evidence suggests that TDP-43 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) are closely related. hnRNP K is an RNA-binding protein that can regulate a host of cellular processes associated with gene expression. Using SH-SY5Y cells, TDP-43 accumulates in stress granules in response to mitochondrial inhibition induced by sodium arsenite or paraquat, and this is controlled by kinases such as c-Jun N terminal kinase (JNK). The co-localisation of TDP-43 with stress granules could be prevented by inhibition of JNK. In addition, JNK inhibition fully blocked both TDP-43 and hnRNP K stress granule accumulation. Using NSC-34 cell lines expressing cherry tagged human WT TDP-43, A315T, or Q331K TDP-43 mutants, phospho hnRNPK levels were evident in cells treated with sodium arsenite expressing WT TDP-43 however, cells expressing Q331K mutant TDP-43 showed complete loss of other RNA binding proteins such as Fused in Sarcoma (FUS), and hnRNP K. This may suggest that WT TDP-43 can regulate the stability of hnRNP K and other RNA binding proteins, while the mutants inhibit this. Dissecting the molecular events resulting in TDP-43 accumulation may facilitate understanding the greater toxicity and proteinopathies associated with mutated TDP-43 in ALS, and FTD patients.

POS-TUE-080

THE DIFFERENTIAL EFFECTS OF RUBROSPINAL TRACT AND RED NUCLEUS LESIONS ON SKILLED REACHING

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We have recently shown that small spinal cord lesions that damage the rubrospinal tract (RST) while sparing the other fibre pathways running in the lateral funiculus selectively abolish the arpeggio movement in skilled reaching (Morris et al., 2011). On the other hand, lesions of the red nucleus (RN) have been shown to interfere with several aspects of skilled reaching including the arpeggio movement (Whishaw and Gorny, 1996; Whishaw et al., 1998). The RST is the main descending output of the RN. Deficits in arpeggio after RN lesions are therefore in line with our recent findings. However, the additional deficits reported after RN lesions are difficult to reconcile with our recent results. The present study was designed to compare, in the same experimental setup, the outcomes of RN lesions with that of lesions to the RST. Long Evans female rats were trained to reach for single sugar pellets. After the completion of the training, the animals were subjected to either RN or RST lesions. Detailed movement analysis revealed that both types of lesions abolish the arpeggio movement. This finding supports the view that the arpeggio movement is under the control of the RST. RN lesions, however, creates additional deficits in the grasping action. The results are explained in terms of the involvement of the RN with a network of neural structures that are directly involved in motor control (e.g.motor cortex and cerebellum). In light of these anatomical considerations, it is not surprising that lesions to the RN have a greater impact on skilled reaching than RST lesions.

POS-TUE-081

DYSREGULATION OF AMPK SIGNALLING ENERGETIC PATHWAYS IN MODELS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Purpose: AMP activated protein kinase (AMPK) is a key metabolic and stress sensor activated under conditions of cellular energy depletion and promotes neurodegeneration in models of Alzheimer's and Huntington's disease. ALS patients and mouse models demonstrate evidence for abnormal energy homeostasis early in disease. We therefore hypothesised that aberrant AMPK signalling contributes to ALS motor neuron degeneration. Methods: AMPK expression level and phosphorylation was examined by Western blotting in (i) neuronal NSC-34 cells stably transfected with normal or ALS-linked mutant; SOD1 or TDP-43 and (ii) spinal cords and brains of transgenic mutant SOD1G93A and TDP-43A315T mice (n=3-5) at pre-symptomatic (30, 60 days) and symptomatic ages (90 days). AMPK sub-cellular distribution was examined using immunocytochemistry and biochemical cell fractionation. Results: We observed that AMPK activity was significantly increased in spinal cords of transgenic SOD1G93A mice at 90 days. In contrast, AMPK activity was significantly reduced in spinal cords of transgenic TDP-43A315T mice at 60 days, as well as in NSC-34 cells stably expressing mutant TDP-43. Confocal microscopy and cell fractionation revealed that active phophorylated AMPK was recruited from the cytoplasm to nucleus in NSC-34 cells treated with AMPK activator AICAR. We observed nuclear exclusion of phospho-AMPK in NSC-34 cells expressing SOD1 or TDP43 mutants, which correlated with nuclear depletion of these ALS-linked misfolded proteins. Conclusion: We demonstrate dysregulation of AMPK pathway signalling in multiple ALS models, arguing for a role of energetic abnormalities in ALS. Interestingly, we observed disruption of nuclear localisation of phospho-AMPK which mirrored mutant SOD1 and TDP-43 nuclear exclusion, suggesting abnormal neuronal nuclear transport in ALS models. The molecular mechanisms underlying nuclear exclusion of AMPK and ALS-linked proteins are currently being investigated.

POS-TUE-082

RESPIRATORY COMMANDS CAN BE CONSISTENTLY TRIGGERED FROM THE PERIAQUEDUCTAL GRAY IN A PERFUSED BRAINSTEM PREPARATION OF RAT

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Purpose: The midbrain periaqueductal gray (PAG) is classically known to be involved in meditation of pain and analgesia, aggression, fear and anxiety, and vocalization. It is a recent discovery that the PAG modulates baseline breathing generated by brainstem circuits to adapt breathing to specific behaviour or emotion. It was shown previously that various types of breathing patterns can be elicited from various sub-compartments of the PAG. However, PAG mediated breathing changes are potentially undervalued in anaesthetised rat preparations. Therefore we investigated midbrain evoked breathing modulations in a decerebrate in situ perfused brainstem preparation. Methods: The baseline breathing pattern was monitored via simultaneous recording of phrenic, vagus and abdominal iliohypogastric (L1) nerve motor activity before and after PAG microinjection (multi-barrelled pipettes) of glutamate (30-50nl, 10mM) and isoguvacine (GABA-receptor agonist), 30-50nl, 10mM). The most potent injection sites were marked with microinjection of Rhodamine beads. Results: In n=7 preparation glutamate microinjection (n=40) triggered a variety of transient breathing changes ranging from phrenic nerve frequency modulation without specific changes in vagal and or abdominal respiratory motor output to specific modulation of either vagal or abdominal activity without change of baseline phrenic discharge. Moreover, at n=15/16 glutamate injection sites which triggered potent respiratory modulation subsequent injection of isoguvacine had no effect on the baseline motor pattern. Conclusions: The lack of isoguvacine effects indicates that the PAG has no role in respiratory pattern generation per se but its excitation still triggers higher respiratory commands in situ. We conclude that the perfused brainstem preparation is a valid model for detailed studies of synaptic mechanisms underlying the processing of higher respiratory commands.

POS-TUE-083

CHARACTERISATION OF THE MUSCLE-MOTOR NEURON TOPOGRAPHY OF THE MOUSE FORELIMB

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Purpose: Our current focus is exploring strategies to deliver therapeutic genes to specific populations of motor neurons. This can be achieved via intramuscular injections of viral vectors and the ensuing retrograde transport of the therapeutic gene into targeted motor neurons. We have previously described the organisation of the motor columns in the rat forelimb (Tosolini and Morris, 2012). With the increasing prevalence of mouse models of motor neuron disease and spinal cord injury, we aimed to define the precise relationship between different forelimb muscles and the motor neurons that innervate them in the mouse. **Methods:** The motor end plates (MEP) of the forelimb were revealed using acetylcholinesterase histochemistry and this information was used to create a motor end plate map. This map was subsequently used as a guide to perform intramuscular injections of retrograde tracer along the entire MEP region of individual forelimb muscles. One week later the animals were intra-cardially perfused and the spinal cords were dissected, sectioned and analysed under epifluorescence. For each muscle, labelled motor neurons were plotted on a spinal cord schematic representation and stacked thereafter to create a motor neuron map. **Results:** This study reveals that mice motor neurons are arranged in columns spanning multiple spinal segments. Individual motor columns have substantial overlap with other motor columns in all axes. **Conclusion:** Both the motor end plate map and the motor column map constitute a valuable guide for the selection of appropriate muscle(s) for the delivery of therapeutic genes into specific motor neurons within the cervical spinal cord. *Tosolini AP and Morris R (2012) Spatial characterization of the motor neurons innervating the rat forelimb. Neuroscience 200:19-30.*.

POS-TUE-084

THE PRECEREBELLAR NUCLEI IN THE C57BL MOUSE - NEW INSIGHTS FROM TRACING AND GENE EXPRESSION

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PURPOSE To make a comprehensive map of the precerebellar (PreCb) nuclei to assist studies of gene expression in the rhombic lip in the C57BL mouse during development. **METHODS** We used retrograde tracing after cerebellar injections of HRP, combined with gene expression data from Wnt1, Math1, and Hoxa3 cre lineages to characterize the mossy and climbing fibre-issuing PrCb goups of the hindbrain. **RESULT** In addition to the classical five precerebellar hindbrain nuclei (pons, external cuneate, reticulotegmental, lateral reticular, and inferior olive) we found that over 30 other hindbrain nuclei, including three previously unreported cell groups, project to the cerebellum. Among the novel mossy fibre projecting nuclei, we have shown that the linear nucleus of the hindbrain is a prominent extension of the lateral reticular nucleus. We have identified a large previously undescribed PrCb group mixed in with the fascicles of the motor trigeminal nerve; we have called it the interfascicular trigeminal nucleus. These neurons were previously thought to supply the tensor tympani muscle. We have in addition identified a group of displaced olivary neurons on the surface of the pyramid in the C57BL mouse (and not in other strains), which we have named the arcuate nucleus. The 'arcuate' neurons in the C57BL mouse express Calb1 and project to the contralateral paraflocculus, and so the nucleus is not homologous with the human arcuate nucleus, which projects Calb1 negative mossy fibres to the cerebellum. **CONCLUSION** The PreCb system in the mouse is more extensive than previously thought, and several of the nuclei have not been previously described at all. These findings provide a platform for further study of the developing rhombic lip.

POS-TUE-085

RESPIRATORY RHYTHM GENERATION CAUDAL TO OBEX

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Purpose: The simple act of drawing air in and out of the lungs is in fact mediated by a complex neuronal network within the brainstem, with the precise neuronal mechanisms of respiratory rhythm generation remaining rather equivocal. Precedence has been given to the pre-Bötzinger complex, labeled the 'rhymogenic kernel' of respiration. The contribution and necessity of the other network areas remains unclear. **Methods:** Here we investigated whether spinalised animals can produce inspiratory rhythm. We recorded spinal inspiratory phrenic (PNA) and cranial inspiratory hypoglossal (HNA) and vagal (VNA) nerve activity in the perfused brainstem preparation of rat. Complete transverse transections were performed at 1.5mm (pyramidal decussation) or 2mm (first cervical spinal segment) caudal to obex. The arterial chemoreflex was elicited via 0.1ml bolus injection of sodium cyanide before and after transection. After the experiment the tissue blocks were cut (50µm) and transections verified histologically (thionine staining). **Results:** Caudal transections (n=15 preparations) immediately eliminated descending network drive for PNA, while the rhythm and discharge pattern of cranial HNA or VNA remained unaffected. Rostral transections (n=10) also abolished PNA immediately, however, HNA or VNA also progressively diminished in amplitude and rhythm. Chemoreceptor activation in 7/10 preparations only triggered tonic, non-rhythmic HNA or VNA, indicating that synaptic afferent input is still mediated within an intact respiratory brainstem circuitry. Histological analysis showed that a putative rhythmogenic region caudal to obex is located caudal to the lateral reticular nucleus, containing a cell population extending from the pyramidal decussation into the upper half of spinal cord segment 1. **Conclusions:** Ascending synaptic inputs arising from a very caudal medullary and high cervical spinal cord area (A1 cell group, retroambiguus?) are essential for maintenance of mammalian respiratory rhythm generation.

POS-TUE-086

GLUCOPRIVATION OF HYPOTHALAMIC NEURONS ELICITS THE COUNTER-REGULATORY RESPONSE TO HYPOGLYCEMIA

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Whether the sympathetic counter-regulatory response to hypoglycaemia is activated by glucose sensing neurons in the fore- or hind-brain remains controversial, since systemic hypoglycaemia excites neurons located in both areas. In this study, we hypothesize that neurons in the perifornical hypothalamus (PeH), but not in the rostral ventrolateral medulla (RVLM), sense the reduction in glycaemia and increase the adrenal sympathetic nerve activity (ASNA) to the chromaffin cells. Electrophysiological experiments were conducted in anaesthetized (urethane, 1.2 g/kg i.v.), paralysed (pancuronium bromide, 1 mg/kg i.v.), and artificially ventilated male Sprague-Dawley rats. Local glucopaenia, induced by bilateral microinjections of 2-deoxy-D-glucose (2DG; 15 ng/50 nl) into the PeH, increased ASNA (101±1% vs 175±11%, N=10) and blood glucose (6.6±0.3 vs 7.6±0.3 mmol/ml, N=9). A higher dose of 2DG (150 ng/50 nl) further increased ASNA (157±7% vs 280±59%, N=6) and blood glucose (7.6±0.3 vs.9.2±0.9 mmol/ml, N=4). Microinjections of 2DG (15 ng/50 nl) into the RVLM (N=6) neither changed ASNA (101±1% vs 84±14%) nor blood glucose (6.4±0.1 vs 6.9±0.1 mmol/ml). On the other hand, subsequent intravenous infusion of 2DG (300 mg/kg) increased ASNA (101±1% vs 183±8%) and blood glucose (6.4±0.1 vs 13.5±0.4 mmol/ml). The results indicate that neurons in the PeH, but not in the RVLM, are glucose sensitive and their excitation produce adrenaline release in response to hypoglycaemia. *Supported by the NHMRC.*

POS-TUE-087

ELECTRICAL STIMULATION ENHANCES RECOVERY OF THE PERISTALTIC REFLEX DURING NICOTINIC BLOCKADE IN GUINEA PIG ILEUM

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Purpose: Enteric neuronal dysfunction has been linked to functional bowel disorders. Acetylcholine acting at nicotinic receptors is primarily responsible for fast synaptic transmission between enteric neurons, however, previous literature suggests that the peristaltic reflex can persist during nicotinic receptor blockade. Our aim was to determine whether electrical stimulation of nerves enhances recovery of peristalsis during nicotinic blockade. **Method:** Segments of proximal ileum (5-7cm) were taken from guinea pigs (391±23g) of either sex and cannulated and pressure recordings were made (anal end). Video recordings were made of intestinal movements and drugs were added to the serosal side of the intestine. Peristalsis was induced by step-wise increases in intraluminal pressure (oral end). The pressure at which four successive peristaltic contractions occurred (peristaltic threshold; PT) were compared in unstimulated versus electrically stimulated (ES; oral end; 1ms duration; 250 pulses, 1Hz) using a t-test. **Results:** Nicotinic receptor blockade with hexamethonium (HEX; 300µM) caused transient inhibition of pressure induced peristalsis (n=16) with an increase in sub-threshold peristaltic contractions over time (n=7 of 16). Electrical stimulation enhanced the recovery during nicotinic blockade with 6 of 22 preparations showing almost complete recovery (6 out of 6 attempts to evoke peristalsis were successful; PT control: 30.9±1.3mmH_2O versus HEX+ES: 40.8±2.2mmH_2O). Twelve showed no recovery but 4 of 22 partially recovered with 2.5 out of 6 attempts successful (PT control: 32.5±2.50mmH_2O versus HEX+ES: 43.3±4.36mmH_2O). Neither the nicotinic antagonist mecamylamine (3µM; n=6), nor a combination of HEX+muscarinic M1 antagonist (VU-0255035, 150nM; n=3) were any more effective then HEX alone. **Conclusion:** Electrical stimulation enhances the recovery of the peristaltic reflex in the presence of nicotinic receptor blockade but M1 receptors are not required for recovery.

POS-TUE-088

ARCUATE NEUROPEPTIDE Y CONTROLS ENERGY EXPENDITURE VIA A PARAVENTRICULAR NUCLEUS RELAY

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Neuropepetide Y (NPY) expressed in the arcuate nucleus (Arc) is best known for its powerful stimulation of food intake. Arc NPY is increased under various conditions including fasting, stress or chronic overfeeding leading to decreased energy expenditure. However, the regulatory mechanisms behind this are unclear. Here we demonstrate that Arc specific re-introduction of NPY into otherwise NPY deficient NPY-/- adult mice is sufficient to trigger a transient but rapid reduction in energy expenditure. This change is accompanied by a marked decrease in brown adipose tissue temperature without any alterations in body weight or adiposity. Mechanistically, a key change induced by Arc NPY signalling is a marked Y1 receptor-mediated reduction in tyrosine hydroxylase (TH) mRNA and protein expression in the hypothalamic paraventricular nucleus (PVN), also associated by reduction in TH expression in the locus coeruleus (LC) and brainstem, suggesting direct control of Arc NPY on sympathetic output and consequently metabolic rate. Consistent with this, Arc NPY signalling reduced serum catecholamine levels and down-regulated ß3 adrenergic receptor expression in brown adipose tissue (BAT), a well-known sympathetically-innervated tissue. Similarly, Arc-only NPY signalling decreased thermogenesis, as indicated by down regulation of uncoupling protein 1 and peroxisome proliferator-activated receptor-γ coactivator α expression in BAT. Taken together, these data demonstrate that the primary role of NPY produced in the Arc may not be to simply increase food intake but in addition control energy balance by conserving energy through reducing energy expenditure via influencing BAT thermogenesis.

POS-TUE-089

DOWN-REGULATION OF AQUAPORIN 1 AND 3 IN THE MUCOSA OF SIGMOID COLON OF PATIENTS WITH SLOW TRANSIT CONSTIPATION

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Purpose: Slow transit constipation (STC) is a clinical syndrome, manifested by extreme difficulty in passing stool. No efficacious treatment has been successfully developed because of its idiopathic nature. Aquaporin (AQP) water channels, identified in recent years in the gastrointestinal tract, are thought to be important in cell volume control and regulation of water flux into and out of the lumen. Altered AQPs could explain some of the pathophysiology of STC, thus, our aim was to examine the expression of AQPs in STC colon. **Methods**: Sigmoid colonic segments were obtained from age-matched patients undergoing resection for STC (23-69 years, n=14), or for carcinoma (controls, 30-68 years, n=22). RT-PCR was used to determine mRNA expression of AQP1-11. Immunohistochemistry of AQP1 and AQP3 was performed to localize in STC and control. **Results**: RT-PCR revealed transcripts for all AQP subtypes in the colon and further real-time PCR demonstrated that AQP1 mRNA was 5.5-fold more abundant in muscle than in mucosa, while other AQPs displayed higher expression in mucosa. There was no differential expression of AQPs in STC muscle compared to control muscle. In contrast, a 1.9-fold (*P*=0.0152, Mann Whitney test) and 1.4-fold (*P*=0.0148) down-regulation of AQP1 and AQP3, respectively, was seen in STC mucosa. Immunoreactivity for AQP1 and AQP3 was mainly present in enteric ganglia, colonic epithelial cells and endothelial cells of blood vessels. In STC, reduced AQP3 immunoreactivity was observed in epithelia and ganglia whereas AQP1 seemed reduced in ganglia mainly. **Conclusion**: Altered AQPs in the mucosa may cause reduction in faecal water content, thus contributing to defecation difficulties in STC patients.

POS-TUE-090

SPINAL PREPROGLUCAGON AXONS PREFERENTIALLY INNERVATE SYMPATHETIC PREGANGLIONIC NEURONS (SPN)

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Purpose: Within the brain, preproglucagon (PPG) neurons, located mainly in the nucleus tractus solitarius and reticular formation, produce GLP-1, a peptide that influences food intake. Brain PPG axons primarily innervate autonomic control areas, consistent with regional GLP-1 receptor expression. GLP-1 receptor mRNA also occurs throughout the spinal cord but the spinal distribution of PPG axons is unknown. Here, we examined this question using mice that express yellow fluorescent protein (YFP) under PPG promoter control. **Methods**: Two-colour immunoperoxidase labelling was done on spinal segments T1 to S3 from 5 male and 2 female YFP-PPG mice. YFP-immunoreactivity was visualized with a black reaction product; and choline acetyltransferase (ChAT)-immunoreactivity, with a brown product. **Results**: Non-varicose, YFP-immunoreactive (IR) axons travelled rostrocaudally in white matter tracts, particularly in the ventral white commissure and around the ventral median fissure. In segments T1-L2, many varicose, YFP-IR axons travelled between the intermediolateral cell column and the central canal, closely apposing ChAT-IR SPN in both locations. In S1 and S2, rare YFP-IR terminals closely apposed ChAT-IR parasympathetic preganglionic neurons. In the ventral horn, occasional ChAT-IR somatic motor neurons also had YFP-IR close appositions. A few YFP-IR neurons were present in lower lumbar and upper sacral segments, mostly within laminae V and VI. **Conclusions**: PPG neurons innervate spinal cholinergic neurons. SPN receive by far the densest GLP-1 innervation. The distribution of spinal PPG axons correlates well with the distribution of spinal GLP-1 receptors. PPG neurons could directly modulate sympathetic outflow through their inputs to SPN.

POS-TUE-091

THE EFFERENT PATHWAY OF THE INFLAMMATORY REFLEX

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INTRODUCTION: The nervous system regulates immune function by an inhibitory action on inflammation. When activated by immune challenge (mimicked here by lipopolysaccharide - LPS), the 'inflammatory reflex' drives sympathetic nerves to the spleen, which act to suppress the release of proinflammatory cytokines such as TNFα by macrophages. The preganglionic link from the CNS to the splenic sympathetic nerves has been proposed to be the 'cholinergic anti-inflammatory pathway', mediated by the vagus (Tracey KJ, Nat Rev Immunol 9, 418-28, 2009), but we recently found no anatomical or electrophysiological evidence that such a neural pathway exists (Bratton BO et al., Exp Physiol 2012, in Press). **PURPOSE:** To test whether conventional sympathetic preganglionic pathways mediate the inflammatory reflex. **METHODS:** Four groups of 5 urethane-anaesthetised rats (1.4 g/kg i.v.) were subjected to either bilateral section of the (preganglionic sympathetic) splanchnic nerves or sham surgery, before being given either intravenous LPS (60 µg/kg) or saline. Blood samples were taken before and 90 mins after LPS, when the spleen was also removed. Spleens and plasma samples were assayed for TNFα by ELISA. **RESULTS:** In saline-treated animals spleen and plasma TNFα levels remained low. In LPS treated animals after sham surgery, plasma TNFα rose to 1722±251pg/ml and spleen TNFα to 1925±234 pg/100mg. The corresponding levels in LPS-treated animals after splanchnic nerve section were 2536±117pg/ml and 2988±70 pg/100mg (both P<0.02 compared with sham). **CONCLUSION:** The splanchnic nerves exert a restraining action on both splenic and plasma TNFα responses to LPS. Sympathetic preganglionic nerves evidently mediate the inflammatory reflex.

POS-TUE-092

EXPRESSION AND FUNCTION OF TOLL-LIKE RECEPTOR (TLR) 3 AND 7 ON VAGAL SENSORY NEURONS

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Background: TLR3 and TLR7 are innate pattern recognition receptors used by immune cells to detect double (TLR3) and single (TLR7) stranded viral RNA and initiate subsequent antiviral immune responses. Many non-immune cells also express TLRs, the functional significance of which is unclear. **Purpose**: To investigate whether vagal sensory neurons express TLR3 and TLR7, and if activation of these TLRs evokes changes in membrane excitability or neuronal growth. **Methods**: Experiments were performed on adult C57BL/6 mice of either sex. TLR expression in mouse vagal nodose-jugular ganglia was assessed by RT-PCR and immunohistochemistry. The effects of the TLR agonists poly I:C (TLR3) and imiquimod (TLR7) on neurite outgrowth and electrophysiological membrane responses were determined in vitro using acutely dissociated cultured mouse vagal sensory neurons. **Results**: Transcripts for TLR3 and TLR7, and their downstream signalling molecules MyD88, IRF3 and IRF7, were all detected in vagal sensory ganglia cDNA (n=3). Cultured vagal sensory neuron soma and neurites immunostained positively for both TLR3 and TLR7 (n=4). In the presence of either poly I:C or imiquimod, neurite outgrowth over 4 days of culture was significantly reduced (P<0.05; n=6 per treatment). Furthermore, in patch clamp electrophysiological studies bath application of poly I:C or imiquimod produced strong inward currents and depolarized the resting membrane potential of all neurons tested (n=5 cells per treatment). **Conclusion**: Vagal sensory neurons express functional TLR3 and TLR7. Activation of these pattern recognition receptors increases excitability and attenuates the growth of vagal sensory neurons. These data suggest TLRs may play important roles in sensory neuronal responses to visceral viral infections.

POS-TUE-093

A NOVEL CONDITIONAL ANTEROGRADE VIRAL TRACING SYSTEM FOR DISSECTING NEURAL CIRCUITS

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Neurotropic viruses are widely used to map neural circuits. We have previously reported a recombinant HSV1-H129 virus for anterograde transynpatic neuronal tracing (McGovern et al J Neurosci Methos 2012). To expand the utility of these tools we are developing a system allowing visualisation of CNS projection patterns of specific neuronal subtypes. **Purpose**: To construct a H129 virus that switches from EGFP to tdTomato expression under the control of Cre-recombinase (Cre). Selective expression of Cre in specific neuronal subtypes will be achieved using a lentiviral expression system. **Methods**: A CMV driven loxP-EGFP-loxP-tdTomato expression cassette was inserted via homologous recombination into the intergenic region between the UL26/26.5 and UL27 genes within the H129 genome. Recombinant virus was plaque purified before being characterised in vitro. A lentiviral bicistronic expression system was constructed initially with a CMV promoter driving Cre expression coupled to EGFP. Lentiviral vectors are designed so that expression of Cre can be under the control of neuronal subtype specific promoters. **Results**: The recombinant-H129 virus demonstrated comparable growth (3x107pfu/ml) and replicated as efficiently as wildtype-H129 in growth replication experiments (n=3). In the absence of Cre, only EGFP expression was observed in H129 infected cells. Cellular expression of Cre using the lentiviral expression system resulted in efficient recombination of the H129 virus thereby enabling robust tdTomato expression. **Conclusion**: Insertion of the loxP-EGFP-loxP-tdTomato expression cassette into the H129 virus had no significant effect on growth and replication. The recombinant-H129 virus demonstrates correct functionality when combined with the lentiviral expression system. This two-part system will be a valuable tool for studying the CNS projection pathways of specific neuronal subtypes in vivo.

POS-TUE-094

PHARMACOLOGICAL ANALYSIS OF SYMPATHETICALLY-MEDIATED CONSTRICTION IN THE MOUSE TAIL ARTERY

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Purpose: We are developing adrenoceptor knockout mice to analyse postjunctional mechanisms involved in sympathetic neurovascular transmission. First we need to define neurovascular transmission in normal mice using pharmacological antagonists. Perivascular stimulation of rat tail artery evokes contractions mediated by α_1- and α_2-adrenoceptors (ARs), acting synergistically [1] whereas, *in vivo*, α_2- but not α_1-AR blockers cause vasodilation, increasing tail skin temperature [2]. **Methods:** Male C57Bl mice (4-6 months) were killed with CO_2 and arterial rings 2 mm long from proximal (2 cm) and distal (5 cm) sites along the tail (~8 cm long) were mounted on myographs. The effects of 100 nM prazosin (α_1-AR antagonist), 100 nM rauwolscine (α_2-AR antagonist) and 1 mM suramin (P2X receptor antagonist) on contractile responses to supramaximal transmural stimuli were examined. **Results:** Peak amplitude of contractions occurred earlier at higher frequencies and were larger proximally. Prazosin reduced the early phase of contraction by ~50% at both sites whereas rauwolscine reduced the later phase, exerting more block at 0.5 Hz (~80%) than at 8 Hz (~50%). Suramin reduced both phases of contraction distally by ~40%, but potentiated the later phase proximally. **Conclusion:** P2X receptors and α1- and α2-ARs are involved in nerve-evoked contractions of mouse tail artery, with responses mediated by α2-ARs dominating at lower frequencies. Unlike in rat, P2X receptor activation inhibits the !α2-AR component in the proximal segment but potentiates it distally. **References:** [1] Yeoh M, McLachlan EM, Brock JA. (2004) J Physiol. 561, 583-596 [2] Redfern WS, MacLean MR, Clague RU, McGrath JC. (1995) Br J Pharmacol 114, 1724-30.

POS-TUE-095

CHEMOTHERAPY INDUCED DIARRHOEA: TARGETING SECRETOMOTOR NEURONS

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Purpose: Common side-effects of anti-cancer chemotherapy include nausea, vomiting, constipation, diarrhoea. Secretomotor neurons responsible for secretion throughout the gastrointestinal tract are located within the intestinal wall in the submucosal plexus. The effects of anti-cancer chemotherapy on these neurons have not been studied and can lead to understanding the mechanisms underlying gastrointestinal side-effects of chemotherapy and development of therapies to combat uncomfortable side-effects suffered by patients undergoing anti-cancer treatment. **Methods:** Anti-cancer chemotherapeutic drug oxaliplatin (3 mg/kg/d) was administered *in vivo* to Balb/c mice intraperitoneally three times a week. Wholemount and cryostat preparations of proximal ileum, jejunum and colon segments were examined immunohistochemically in both oxaliplatin and sham-treated mice at days 3, 7 and 14 following injections. Submucosal neurons and their axons were labelled using anti-Protein Gene Product (PGP) 9.5 antibody. Subpopulations of secretomotor neurons were labelled using antibodies for Vasoactive Peptide (VIP), Neuropeptide Y (NPY) and Somatostatin. Structural damage was assessed histologically. **Results:** Total number of submucosal neurons significantly decreased at day 3 following oxaliplatin injection. The number and proportion of NPY and VIP-immunoreactive neurons significantly decreased at days 3, 7 and 14 following oxaliplatin treatment. No morphological changes in neurons were observed in wholemount preparations; however a noticeable decrease in axonal density across all time points in oxaliplatin-treated mice was displayed. Histological assessment uncovered prominent damage to intestinal mucosa, the most severe at days 3 and 7. **Conclusion:** This study is the first to examine the effects of oxaliplatin on the secretomotor neurons. Results demonstrated that repeated exposure to oxaliplatin causes significant neuronal loss and decreased proportions of neurons immunoreactive to NPY and VIP, which may be a contributing factor to functional changes within the gut.

POS-TUE-096

LITHIUM CHLORIDE REDUCES RESPIRATORY RATE IN RATS: A NEW APPROACH FOR STUDYING EMESIS?

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Purpose: Preclinical studies of emesis are held back by the lack of relevant physiological indices and by lack of vomiting reflex in most laboratory rodents. Based on our recent observation that motor activity is highly correlated with respiratory rate in rats (Kabir, Physiol Behav. 2010, 101:22-31), we tested the hypothesis that pro-emetic interventions would affect their respiratory pattern. Methods: Using whole-body plethysmography, we recorded respiration in 6 adult male Wistar rats after i.p. administration of either the prototypic emetic agent LiCl (20 mg/kg) or Ringer's solution. Results: Loss of motor activity (and of associated increases in respiratory rate) was quite obvious starting from 2-3 min post-drug. Post-LiCl, respiratory rate was significantly lower (126±9 vs. 178±10 cpm, $p<0.005$) and tended to be less variable (62±4 vs. 73±3%; $p=0.07$) compared to the post-Ringer condition. Furthermore, while median values of respiratory rate did not differ between the treatments (ie most of the time animals were breathing at the same frequency), LiCl reduced the fraction of time spent at relatively high respiratory rate (>200 cpm) from 25±3% (post-Ringer) to 9±2% ($p=0.004$). Thus, reduction of the mean respiratory rate by LiCl was predominantly due to reduced contribution of higher-frequency breathing that is normally associated with motor activity and/or arousal. Pre-treatment antiemetic ondansetron (50 mg/kg) prevented respiratory changes induced by LiCl. Conclusions: Providing that effects observed in our study were quite robust and sensitive to antipemetic drug, we suggest that simple and non-invasive respiratory monitoring may be a promising approach for studying emesis in rodents.

POS-TUE-097

MECHANISMS UNDERLYING THE EFFICACY OF THE ADJUSTABLE GASTRIC BAND – INSIGHTS FROM A RODENT MODEL

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Currently, bariatric surgery remains the only effective treatment for morbid obesity. Laparoscopic adjustable gastric banding (LAGB) is a commonly performed bariatric procedure, particularly outside the USA: however, the mechanism(s) underlying its efficacy are unclear. This study aims to elucidate the role of sensory neural pathways in mediating AGB-induced satiety in a rodent model and assess the effectiveness of adjuvant therapies on AGB induced weight loss. Adult male Sprague Dawley rats (n=8/group) were fitted with an AGB, just below the gastro-oesophageal junction. Our previous data indicate that inflation of the band causes an increase in numbers of Fos-positive neurons in the rostral division of the medial NTS. This could be attributed to a neural, a neural - humoral or a direct humoral link. To test this, capsaicin (cap) was used to ablate vagal sensory fibres using CCK- induced anorexia as a biomarker of the extent of the lesion. Cap treatment resulted in a diminution of the acute and chronic effects of AGB on activation of NTS neurons and an amelioration of the AGB – induced reduction in food intake, body weight gain, fat mass and feed efficiency ($p<.05$). Associated with this reduction in body weight and fat mass in obese rats, there is a reduction in energy expenditure that can be effectively ameliorated by co-treatment with factors such as thyroid hormone, which increase energy expenditure in brown adipose tissue ($p<.05$). These data support the hypothesis that LAGB exerts its effects via the modulation of both, neural and hormonal pathways. Adjuvant therapies that increase energy expenditure can enhance the effectiveness of the AGB.

POS-TUE-098

EFFECTS OF ANTI-CANCER CHEMOTHERAPY ON GASTROINTESTINAL IMMUNITY AND NEURO-IMMUNE INTERACTIONS

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Purpose: The efficacy of anti-cancer chemotherapeutic treatment is constantly challenged by the side-effects associated with it: nausea, vomiting, diarrhoea, malnutrition. Anti-cancer chemotherapy causes damage to the enteric nervous system (ENS) responsible for gastrointestinal functions such as motility, secretion and the absorption of nutrients. It is unclear whether ENS damage is a direct toxic effect of anti-cancer chemotherapeutics, or induced by indirect mechanisms. This study investigates the immune response and neuro-immune interactions during anti-cancer chemotherapeutic treatment with oxaliplatin. **Methods:** Balb/c mice received oxaliplatin (3mg/kg/d) intraperitoneally three times a week for three weeks. Mesenteric lymph nodes and Peyer's patches (n=5/mouse) from sham and oxaliplatin-treated mice were harvested at days 3, 7, 14, 21. To analyse the immune response, fluorescence-activated cell sorting analysis was conducted to quantify the number and type of immune cells. Immunohistochemistry of the ileum (with Peyer's patches intact) and colon allowed for qualitative analysis of neuro-immune interactions. Neurons were labelled with anti-β-Tubulin and immune cells were labelled with antibodies specific to each type. **Results:** A significant increase in the total number of natural killer cells, natural killer T-lymphocytes and γδ T-lymphocytes in mesenteric lymph nodes observed at day 3 which was reduced by days14 and 21 (n=5 mice/group/timepoint). Experimental data showed that oxaliplatin treatment induces an increase in γδ T-lymphocytes within the gastrointestinal wall at day 3. Immunohistochemical assessment showed γδ T-lymphocytes in close proximity to enteric neuronal processes projecting to the mucosa (n=5). **Conclusion:** This study is among the first to examine immune response and neuro-immune interactions in the gastrointestinal tract during anti-cancer chemotherapy. Results indicate that oxaliplatin potentiates immune responses, which may lead to aberrant neuro-immune interactions and eventual damage to enteric neurons.

POS-TUE-099

ANATOMY AND FUNCTION OF ROSTRAL VENTROLATERAL MEDULLA (RVLM) NEURONS THAT PROJECT TO THE CONTRALATERAL BRAINSTEM

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Purpose: Previous experiments indicate that the contralateral brainstem is a source of ongoing drive to RVLM sympathetic premotor neurons, but the identity of neurons responsible are unknown. This study examines distribution, phenotype, behaviour and post-synaptic targets of contralaterally projecting RVLM neurons. **Method1:** Contralaterally projecting neurons were identified by a combined retrograde-anterograde tracing strategy. Latex beads and dextran were co-injected into the RVLM of ketamine/medetomidine anaesthetised rats (n=12). Spinal cholera toxin β-subunit (CTB) microinjections identified bulbospinal neurons in the same animals. 2-4 days later rats were anaesthetised, perfused and fixed. Coronal brainstem sections were examined for tyrosine hydroxylase and CTB immunoreactivity and tracer transport. **Method2:** Antidromic action potentials were evoked by stimulation of the contralateral brainstem in 37 RVLM neurons from 19 urethane-anaesthetised rats. In each case a spike-triggered average of simultaneously recorded splanchnic nerve activity (SNA) was generated. **Results1:** Many RVLM neurons were retrogradely labelled with beads, but few catecholaminergic (12%) or bulbospinal (6%) neurons were labelled. Anterograde labelling was extensive: putative appositions between dextran-labelled terminals and sympathetic premotor neurons were identified in many cases. **Results2:** Antidromically activated neurons were inactive (n=9) or had spontaneous respiratory (n=13) or non-respiratory (n=15) firing. Correlations between neuronal firing and SNA were only seen in non-respiratory neurons (6/15). There was little evidence of barosensitivity in antidromically activated neurons. **Conclusions:** While RVLM neurons with projections to the contralateral brainstem are widespread, most are phenotypically and functionally distinct from sympathetic premotor neurons. As we have identified apparent synaptic contacts on sympathetic premotor neurons from the contralateral RVLM and strong correlations between the activity of contralaterally projecting neurons and SNA, this population likely represents a group of sympathoexcitatory interneurons.

POS-TUE-100

HYPOTHALAMIC INPUT TO LOCUS COERULEUS NEURONS IN THE RAT: AN OREXINERGIC CONNECTION

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Purpose: The dorsal pontine locus coeruleus (LC) is densely packed with orexin 1 receptors (OX_1-R) and receives a substantial orexinergic input. In this study, the effects of i.c.v. administration of an OX1-R antagonist on lateral hypothalamic (LH) stimulation-induced activation of LC neurons were examined. **Methods:** Male Sprague Dawley rats were used in all experiments and these were anaesthetized with isoflurane (1.7-1.9%) followed by urethane (1.3-1.4 g/kg, i.v.). Recordings were made from LC neurons in response to stimulation of the LH region. **Results:** Electrical stimulation of the LH (0.5 Hz, twin 0.5 ms pulses, 3 ms interpulse interval, 300μA) evoked excitatory responses in all LC neurons studied (n = 24) as judged by construction of peri-stimulus time histograms. LC neurons responded to LH stimulation with an onset latency of 6.5±0.4 ms and a peak latency of 15.3±0.8 ms. Administration of the OX_1-R antagonist SB334867A (10 nmol, i.c.v.) but not vehicle inhibited LH stimulation-induced activation of LC neurons by 58±14% ($P < 0.05$; n = 6 neurons). Abolition of LH stimulation-induced excitatory responses in LC neurons occasionally (n=3/6) revealed constant latency antidromic responses verified by positive collision tests. **Conclusion:** These findings indicate that the LC receives a robust excitatory input which is mediated, at least in part, by orexin acting at OX_1-Rs. Furthermore, some LC neurons that receive an orexinergic input also project to the LH region. LH-LC connections may be important for maintenance of vigilance during ingestive behavior.

POS-TUE-101

POLYSIALIC ACID IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) IS A SIGNALING MOLECULE IMPORTANT IN CARDIOVASCULAR FUNCTION

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PURPOSE: Glycans (sugar moieties) are post-translational modifications on proteins. The glycan, α2,8-linked polysialic acid (PSA), has a restricted distribution within the brain but is densely concentrated in the NTS, the primary site of termination of cardiopulmonary afferents. The aim was to determine if glycan modification within the NTS or other brain regions alters tonic or reflex cardiovascular function. METHODS: Electrophysiological recording of blood pressure (BP), heart rate (HR) and sympathetic (SNA) nerve activity in urethane anesthetized rats was conducted before and after microinjections of - neuraminidase (NEU), β -Galactosidase (β-GAL) and Peptide-N-Glycosidase F (PNGase-F) - made into the NTS, rostral and caudal ventrolateral medulla. Baroreceptor reflexes were tested before and after enyzme injection. RESULTS: NEU (n=8) compared to vehicle (n=7) evoked significant increases in BP (22.4±5vs3.3±1mmHg), HR (41±10vs-18±4bpm) and SNA (32±12.6vs-10±6%). Furthermore, sympathetic baroreceptor reflexes were significantly attenuated or eliminated. In contrast, β-GAL (n=3) or PNGase-F (n=3) failed to evoke any changes in BP, HR, SNA or reflex function. CONCLUSIONS: NEU, β-GAL and PNGase-F are enzymes that cleave sugar residues differently. NEU removes sialic acid residues from the termini of all membrane attached sugars. The results suggest that PSA acts as a suppressor molecule. Cleavage of PSA in the NTS reduces transmission of vagal afferent information and elevates BP, HR and SNA. β-GAL and PNGase-F act on N and O-linked sugars on glycoproteins The results indicate that sialylation but not glycosylation in the NTS regulates BP, HR and SNA. Microinjection into other areas is underway (RVLM/CVLM).

POS-TUE-102

NEUROTRANSMITTER SYSTEMS ASSOCIATED WITH MENTAL HEALTH DISORDERS: AUTONOMIC CONSEQUENCES

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Mental health disorders (MHDs) are characterised by altered mood and behaviour and are associated with dysfunction of the frontal brain regions including the medial prefrontal cortex (mPFC) and nucleus accumbens (Acb). Cardio-respiratory dysfunction is comorbid with MHDs however understanding the link between these conditions is unclear. The cholinergic or monoamine theories of mood disorders suggest that changes to the levels of relevant neurotransmitters cause the behavioural symptoms evident in MHDs. **Purpose:** to determine if altering the levels of Ach or 5-HT in the mPFC and Acb can influence cardiovascular, respiratory and/or metabolic activity. **Methods:** Ach, 5-HT and receptor selective agonists were microinjected into various regions of the mPFC and Acb in urethane-anesthetised, pancuronium-paralysed and artificially ventilated rats. **Results:** Ach into the mPFC (n=4) decreased arterial blood pressure (AP) with the greatest declines observed in cingulated cortex area 1 (Cg1) (37 ± 3mmHg; vehicle 0.37 ± 1.9mmHg) and prelimbic cortex (PrL) (32 ± 0.6mmHg; vehicle 0.68 ± 1.3mmHg). 5-HT into the mPFC (n=4) also decreased AP with the greatest falls again seen in Cg1 (36.85 ± 4.178mmHg) and PrL (16.84 ± 4.9mmHg). Changes were also seen in splanchnic sympathetic nerve activity, expired CO2 and brown adipose tissue temperature for Ach and 5-HT as well as changes to phrenic nerve activity with Ach only. In the Acb core region little to no response was elicited by any drug in any of variables measured. **Conclusion:** by independently altering the levels of two neurotransmitters (Ach and 5-HT) in two brain regions implicated in MHDs, changes in cardiovascular, respiratory and metabolic activity can be generated suggesting the possibility that alterations in neurotransmitter systems, evoking behavioural changes, may alter the susceptibility to cardio-respiratory disorders.

POS-TUE-103

SEX DIFFERENCE IN THE EXPRESSION OF OESTROGEN RECEPTOR ALPHA WITHIN NORADRENALINE NEURONS IN THE SHEEP BRAINSTEM

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Purpose: There is a sex difference in the feedback actions of oestrogen in the brain to regulate gonadotrophin releasing hormone (GnRH) secretion, exerting a positive feedback action in females and a negative feedback action in males. The neural basis for this is unknown. Previous work in female sheep suggests that noradrenaline neurons in the A1 region of the brainstem are important for the positive feedback actions of oestrogen, but the role of these neurons in males is unknown. We hypothesised that there would be a sex difference in the number of brainstem noradrenaline neurons that express oestrogen receptor alpha (ERα). **Methods:** Dual label fluorescent immunohistochemistry was used to label ERα-immunoreactive (-ir) and dopamine-β-hydroxylase (DBH, a marker for noradrenaline synthesis)-ir cells in the brainstem of rams and ewes (n=6/sex). **Results:** ERα –ir cells were found within the A1 and A2 regions, with regional variation within these nuclei and greater numbers in the ewe (both $P<0.05$), and a region x sex interaction in the A2 region. The proportion of ERα-ir cells that colocalised with DBH varied with region within both the A1 and A2 nuclei, with a greater proportion in ewes compared with rams (all $P<0.05$). **Conclusion:** This study has demonstrated that there is a sex difference in the expression of oestrogen receptors within noradrenaline neurons in the caudal brainstem of the sheep. This may, in part, explain the sex difference in the actions of oestrogen on GnRH secretion in this species.

POS-TUE-104

NRG1 MUTATION ALTERS CYTOKINE RESPONSES RELEVANT TO SCHIZOPHRENIA

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Purpose: Gene-immune interactions are hypothesised to contribute to schizophrenia disease development. With Neureulin-1 (Nrg1) a candidate vulnerability gene and altered cytokines levels recently reported in human Nrg1 mutants; we investigated the peripheral and central cytokine response of Nrg1 heterozygous mutant (Nrg1-Het) mice following an immune stimulus. **Methods:** We treated adult Nrg1-Het and wild type littermates (WT) with melanoma cells, established to induce a chronic immune response, for 9 days (n>8 per group). Cytokine levels were measured in the plasma (n>6 per group) and the brain (n>3 per group) using Luminex or Western blot. Gene expression of signalling molecules was measured using RT-qPCR (n>3 per group). Significance accepted at $p<0.05$. **Results:** We found higher plasma G-CSF and IL-6 levels in Nrg1-Het immune challenged compared to WT challenged mice. Lower levels of G-CSF were found in the hippocampus of challenged Nrg1-Het compared to WT mice. Further, 55% lower IL-6 was found in the prefrontal cortex of Nrg1-Het challenged compared to Nrg1-Het unchallenged mice. In unchallenged mice, Nrg1-Hets had lower levels of phosphorylated AKT protein in the hippocampus. Further, AKT1 mRNA expression was lower in the prefrontal cortex while SOCS3 mRNA expression was higher in Nrg1-Het mice compared to WT. In immune challenged animals, JAK1 mRNA expression was lower in the hippocampus of Nrg1-Het compared to WT mice. **Conclusion:** This study demonstrates that Nrg1 mutation alters IL-6 and G-CSF in the periphery and the brain following an immune challenge. It further shows alterations in AKT, SOCS3 and JAK1 in the brain, key shared signalling pathway molecules. Together these data indicate interactions between Nrg1 mutation and immune challenge can affect signalling, which may alter neuronal activity in schizophrenia-relevant brain areas.

POS-TUE-105

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

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Purpose: Leptin promotes negative energy balance by suppressing energy intake (EI) and elevating energy expenditure through its central action. Central leptin resistance is a hallmark of diet-induced obesity (DIO). Oral administration of teasaponin (TS) can reduce body weight and fat mass. However, little is known if TS has benefit effect in improving central leptin sensitivity. This study investigated the effects of TS on central leptin sensitivity and leptin signalling in the hypothalamic arcuate nucleus (Arc) of DIO mice. **Methods**: After 15 weeks of high-fat diet, DIO mice (n=40) were divided into two groups received either intraperitoneal (ip) injection of TS (10mg/kg, daily) or saline for 20 days. Another group of mice was fed low-fat diet (LF) as control (n=20). Then both DIO mice and LF mice were given intracerebroventricular (i.c.v.) injection of leptin or saline. Food intake and pSTAT3 level in the Arc in response to central leptin administration were assessed. **Results**: TS significantly decreased body weight gain (-210%, p<0.001) and average EI (-24%, p<0.001) of DIO mice. In LF mice, i.c.v. injection of leptin significantly suppressed food intake compared to saline injection (-29%, p=0.001). However, central leptin sensitivity was blunted in DIO mice evidenced by an incapable of suppressing food intake compared to control mice. Importantly, TS treatment reinstalled leptin sensitivity as seeing a significant decrease in food intake after i.c.v. leptin injection for 24 hours (-39%, p=0.023). With TS treatment, pSTAT3 level in the Arc also increased in response to central leptin injection compared to saline group. **Conclusion**: This study suggested that teasaponin can correct central leptin resistance in DIO mice via improving leptin-pSTAT3 signalling in the hypothalamus.

POS-TUE-106

ACUTE AND CHRONIC ACTIVATION OF RELAXIN-3 RECEPTORS (RXFP3) IN THE HYPOTHALAMUS: EFFECTS ON HPA AXIS ACTIVITY IN MICE

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Purpose: The neuroanatomical distribution of relaxin-3/RXFP3 networks in rat and mouse brain suggests they represent an ascending arousal system which is closely linked to CRF stress circuits. Recent studies in rats described activation of the HPA axis by acute icv infusion of relaxin-3, but whether this occurs in mice is unknown. This study explored the hypothesis that acute and chronic administration of a selective RXFP3 agonist into the PVN activates the HPA axis in mice. **Methods:** For acute RXFP3 activation studies, mice (n=26) were surgically implanted an iPVN guide cannula (unilateral), allowed to recover, and injected with either aCSF or 0.1 nmol of a specific RXFP3 agonist ('RXFP3-A2') prior to culling. For chronic RXFP3 activation studies, a lenti-R3/I5-GFP viral construct which encodes transduced neurons to secrete the selective RXFP3 agonist 'R3/I5' and GFP were bilaterally injected into the PVN, and mice were culled 2 weeks later and compared to lenti-GFP control virus or aCSF control groups. **Results:** Mice acutely infused with RXFP3-A2 displayed a strong trend for increased plasma corticosterone, and displayed a statistically significant (p<0.05) increase in cFos within the PVN, compared to control groups. Similar activation of the HPA axis was not observed in mice infused with the lenti-R3/I5-GFP virus, nor were differences in bodyweight observed. However, the time course of GFP expression was characterised (peaking 2-4 weeks post infusion), which paves the way for future studies. **Conclusion:** These studies reveal that relaxin-3/RXFP3 signalling is able to modulate the HPA axis stress response in mice.

POS-TUE-107

NEONATAL LPS EXPOSURE ALTERS ENDOCRINE AND INFLAMMATORY PAIN RESPONSES

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Neonatal exposure to Lipopolysaccharide (LPS) has been linked to altered neuroimmune and endocrine responses. However, little is known about its impact on pain. **Purpose**: To investigate the behavioural, endocrine and neuronal changes in response to neonatal exposure to LPS. **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 PND 7 and 22, rats received an injection of 0.5% and 1.1% formalin (respectively) into the hindpaw. Flinching and licking behaviours were recorded for one-hour post formalin injection. After one-hour, trunk (PND 7) or cardiac blood (PND 22) was collected to assess corticosterone responses and transverse spinal cord slices (300µm thick) were prepared for whole-cell patch-clamp recording (KCH_3SO_4-based internal) from SDH neurons. **Results**: At PND 7, no significant differences were observed in either flinching or licking between rats subjected to LPS (n = 8) or saline (n = 8). At PND 22, LPS-treated rats (n=14) displayed more flinching and licking compared to saline group (n=13), p < 0.05. LPS-challenged rats exhibited elevated corticosterone responses at PND 7 and PND 22 (both p < 0.01). At both PND 7 and PND 22, intrinsic properties of SDH neurons did not differ between saline and LPS-treated rats (n = 31 and 32 respectively for PND 7; n = 35 and 43, respectively for PND 22). Discharge of action potentials remained similar between the two groups at both ages examined. **Conclusions**: Neonatal LPS exposure alters HPA axis activity in preadolescent and neonate rats. This was associated with increased behavioural responses to formalin in preadolescents but not in neonates. These behavioural changes were not accompanied by changes in selected properties of SDH neurons.

POS-TUE-108

PROBDNF REGULATES CHOLESTEROL SYNTHESIS AND MYELINATION BY SCHWANN CELLS

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Background: The neurotrophins exert profound and complex influences upon peripheral nervous system development and myelination. We have recently identified that the neurotrophin BDNF exerts contrasting influences upon peripheral myelination, promoting and inhibiting myelination of distinct subsets of peripheral neurons. Here we investigate whether the precursor form of BDNF, proBDNF, exerts an influence upon peripheral myelination. **Results**: Comparative analysis of the Schwann cell transcriptome following treatment with either mature BDNF or proBDNF in vitro reveals that proBDNF significantly upregulates multiple genes in the cholesterol biosynthesis pathway. We identified this resulted in a physiological response, as quantitative analysis revealed that proBDNF significantly increased total cholesterol levels in Schwann cell cultures (n=3). As cholesterol is a major constituent of myelin, we investigated whether proBDNF also influenced Schwann cell myelination *in vitro*. These data reveal that proBDNF promoted Schwann cell myelination, even on the subset of neurons that BDNF exerts an inhibitory effect (n=3). Importantly, we found that shRNA mediated knockdown of p75NTR in Schwann cells abolishes both the proBDNF induced upregulation of cellular cholesterol and promotion of myelination (n=3). We are currently investigating the influence that proBDNF exerts on peripheral myelination *in vivo*. **Conclusions**: Our results suggest that proBDNF exerts a key role in regulating the availability of cholesterol for incorporation into peripheral nervous system myelin, and that p75NTR plays a critical role in this process.

POS-TUE-109

BDNF PROMOTES OLIGODENDROCYTE MYELINATION *IN VITRO* VIA ACTIVATION OF FYN AND ERK KINASES

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Background Kinases transmit intracellular signals and control complex cellular processes. We have identified that the promyelinating influence that BDNF exerts on oligodendrocytes *in vitro* is dependent on the kinases Erk1 and Erk2. Interestingly, an unrelated Src family kinase, Fyn, is required for oligodendrocyte differentiation *in vitro* and myelination *in vivo*. Here we hypothesise that Fyn is an intermediate kinase that BDNF utilises to activate Erk. **Results** We have previously shown that BDNF promotes oligodendrocyte myelination, through utilizing an in vitro model of myelination consisting of co-cultures of oligodendrocyte precursor cells and dorsal root ganglion neurons. Analysis of co-culture lysates reveals that BDNF stimulates Fyn autophosphorylation (n=3) which appears to be a TrkB dependent effect, as shRNA-mediated knockdown of TrkB in oligodendrocytes prevented the Fyn autophosporylation. Importantly, we show that the promyelinating influence of BDNF is abrogated in the presence of PP2, a pharmacological inhibitor of Src family kinases. Immunohistochemical and Western blot analysis of the co-cultures reveals that PP2 reduced the expression of myelin proteins, inhibited Fyn autophosphorylation and reduced Erk1/2 phosphorylation (n=3). To provide additional mechanistic insight, we stimulated oligodendrocytes that express a kinase dead mutant of Fyn, with BDNF and found that Erk activation was attenuated. This suggests that Fyn is required for Erk activation in oligodendrocytes. Finally, we show that Fyn immunoprecipitates with the full length TrkB receptor, but not with the truncated TrkB isoform. **Conclusions** We propose that BDNF activation of oligodendrocyte-expressed TrkB receptors stimulates association with and phosphorylation of Fyn, which leads to Erk phosphorylation and stimulation of the myelin program.

POS-TUE-110

UNDERSTANDING LINEAR ARRAYS OF MYELINATING CELLS IN THE CENTRAL NERVOUS SYSTEM

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Myelination is critical for rapid conduction of action potentials in the vertebrate nervous system. In the central nervous system (CNS), this function is undertaken by oligodendrocytes (OLs), which are typically arranged in linear arrays along fibre tracts. The mechanisms underlying the establishment of OL arrays during development and adulthood and their relevance to patterns of myelination have not been described. **Purpose**: We aimed to test the hypothesis that *in situ* proliferation of oligodendrocyte progenitor cells (OPCs) is the principal mechanism responsible for the generation of linear arrays. **Methods**: We assessed both birth date and clonal relationship between individual cells within linear arrays in the corpus callosum during normal postnatal development and following regeneration of OLs after cuprizone-induced demyelination (n=4-6/grp). Corpus callosi of *C57Bl/6* mice were analysed at different postnatal time-points to define array density, cellular composition and segmental arrangement by immunohistochemistry. In order to assess the clonal relationship between cells within linear arrays we utilised female heterozygous *XXLacZ* mice that possess one copy of an X-linked *LacZ* transgene. **Results**: Linear arrays were abundant by postnatal day P14 but rarely observed at P7. Analysis of adult *XXLacZ* mice revealed more clonally-derived (all blue or all white) arrays than would be expected by chance alone. Assessment of mice following remyelination revealed efficient regeneration of clonally-derived linear arrays. **Conclusion**: Our data indicate that OL arrays are generated between P7 to P14 reflecting the onset of myelination. Clonal expansion by local proliferation of OPCs likely contributes to their generation and this process is recapitulated during remyelination. We define a novel and generic mechanism for oligodendrogenesis in white matter.

POS-TUE-111

RECEPTOR-MEDIATED GENE TRANSFER STIMULATES AN IMMUNE RESPONSE IN MICROGLIA

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Purpose: Cationic polymers such as polyethyleneimine (PEI) are considered a safer alternative to viruses for gene delivery. We are developing a non-viral vehicle based on PEI and conjugated it to monoclonal antibody OX42 ('OX42-immunogene') for receptor-mediated gene transfer into microglia via CD11b/CR3 (complement receptor 3). This *in vitro* study aimed to assess transfection efficiency and specificity of the OX42-immunogene for microglia and whether this vehicle stimulates unwanted effector functions including production of reactive oxygen species (ROS) and CR3 exocytosis. **Methods:** Mixed glia cultures from neonatal Sprague-Dawley rats were transfected with enhanced green fluorescence protein (EGFP) delivered by either PEI (n=6 transfections) or OX42-immunogene (n=4 transfections). Astrocytic (GFAP) and microglial (Iba1) markers were detected with immunofluorescence. Aggregation of OX42-immunogene was studied with Dynamic Light Scattering (DLS, n=3). ROS production was measured with DCFDA (dichlorodihydrofluorescein) in a 96-well plate (n≥4). Exocytosis of internal CR3 was visualized with OX42 antibodies differentially tagged with fluorophores to detect internal and membrane CD11b (n=3 experiments, n≥90 cells). **Results:** OX42-immunogene markedly reduced the amount of transfected astrocytes compared to PEI, but EGFP expression levels in microglia were very low. The OX42-immunogene formed large aggregates suggesting phagocytosis as the internalization mechanism. Aggregated OX42-immunogene triggered exocytosis of CR3 and ROS production via CR3 and/or Fc-receptors (FcR). **Conclusion:** Aggregation of the OX42-immunogene facilitates cross-linking of immune receptors that activate microglia causing destruction of the non-viral vehicle. FcR may be involved in phagocytosis of OX42-immunogene, because effector functions in peripheral macrophages are not stimulated by CR3 alone. Thus, a second generation immunogene using OX42-F(ab')$_2$-fragments may successfully deliver genes into microglia via CR3.

POS-TUE-112

MINOCYCLINE INHIBITS MICROGLIAL MACROPORE FORMATION IN RESPONSE TO BOTH P2X4 AND P2X7 RECEPTOR ACTIVATION

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Purpose: Microglia, the brain's resident immune cells, are activated by various stimuli including ATP released from damaged CNS. Inappropriate activation of microglia may contribute to various CNS pathologies via chronic release of inflammatory cytokines, BDNF and cytotoxic mediators. The drug minocycline inhibits microglial activation in vivo, but its mechanism of action is not clear. We therefore investigated whether minocycline can block an early event in microglial activation, namely formation of membrane pores (macropores) that allow influx and efflux of large molecules. **Methodology:** Neonatal rat microglia isolated from mixed cultures were placed in Krebs HEPES buffer and treated with substances to induce macropore formation via P2X4 receptors (50μM, 100μM ATP), P2X7 receptors (100μM BzATP) or both (1mM ATP) in the presence and absence of minocycline. Macropore formation was monitored via uptake of the fluorescent DNA binding dye Yo-Pro-1 and confocal microscopy. **Results:** Activation using 100μM ATP to target P2X4 receptors caused macropore formation in 92% of microglia tested (n=52), whereas activation using 1mM ATP caused macropore formation in only 24% of cells (n=27). Similarly the P2X7 agonist BzATP caused activation in only 33% of microglia (n=40). In contrast, almost 100% of cells (n=41) formed macropores when treated with 1mM ATP in a low divalent cation solution, suggesting divalent cations inhibit formation of macropores via P2X7, but not P2X4 receptors. Pore formation could be prevented under all conditions by minocycline. None of the treatments caused significant cell death during the time frame of the experiments. **Conclusion:** Macropore formation in microglia occurs through different mechanisms in response to P2X4 and P2X7 receptor activation, but both of these mechanisms are blocked by minocycline.

POS-TUE-113

CORRELATING CLINICAL READOUTS WITH PATHOLOGICAL AND REGENERATIVE CONSEQUENCES OF SCHWANN CELL APOPTOSIS

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Purpose: Schwann cells (SCs) produce the myelin that insulates peripheral nerves for efficient axonal propagation, neuro-protection and trophic support. Peripheral neuropathies commonly involve demyelination and SC loss. However the extent to which the degenerative and regenerative sequelae following SC loss are generic or disease-specific remains poorly understood. **Methods**: To answer this question, we have created an inducible model of SC apoptosis. Transgenic mice expressing diphtheria toxin receptor regulated by the myelin basic protein promoter (MBP-DTR[25]mice) express DTR in SCs, rendering these cells sensitive to DT-mediated apoptosis. **Results:** Following one intraperitoneal (i.p.) injection of 10ng/g DT, we observed apoptosis in the sciatic nerve of MBP-DTR[25]+DT mice but not control wild-type (WT)+DT mice at 25 days post-DT ($P<0.05$, n=4 per group). Apoptotic cells were immunoreactive for the SC marker S100β. MBP-DTR[25]+DT mice developed hindlimb weakness which peaked by ~25 days post-DT and rapidly recovered by ~28 days. To elucidate the mechanisms of rapid recovery, MBP-DTR[25]+DT and WT+DT mice were given 4xi.p. injections of EdU for 3 consecutive days prior to perfusion fixation 7 days and 28 days post-DT-challenge. In MBP-DTR[25]+DT mice, there was a significant increase in EdU+ cells compared with WT+DT mice at 28 days post-DT ($P<0.05$, n=8 per group). Many EdU+ cells co-expressed the SC lineage marker Sox-10. Ultrastructural analysis revealed evidence of remyelination at 28 days post-DT, although a subset of axons remained demyelinated. **Conclusion:** Our data indicate that targeted SC ablation induces clinical dysfunction followed by rapid recovery. Schwann precursor cells proliferate and differentiate into myelinating SCs resulting in partial remyelination that correlates with functional recovery.

POS-TUE-114

CHRONIC STRESS INDUCES PROFOUND STRUCTURAL ATROPHICATION OF ASTROCYTES WITHIN THE PREFRONTAL CORTEX: A CHARACTERIZATION OF THE RELATIONSHIP BETWEEN ASTROCYTE MORPHOLOGY, DENSITY AND S100β

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Chronic stress is well recognised to decrease the number of astrocytes within the prefrontal cortex (PFC). Indeed, this effect has now been incorporated into theoretical accounts of how stress provokes changes in PFC-dependent behaviour. Recent findings, however, have suggested that our understanding of how chronic stress alters astrocytes may be incomplete. Specifically, it has been shown that chronic stress induces a unique form of microglial hyper-ramification. Whether astrocytes undergo an equivalent form of structural remodelling has not yet been investigated. Such remodelling may be particularly significant given the role of astrocytes in modulating synaptic function. Accordingly, in the current study we examined changes in astrocyte morphology following exposure to chronic stress using three-dimensional digital reconstructions of astrocytes. Our analysis indicated that chronic stress produced profound atrophication of astrocyte process length, branching and volume. We additionally examined changes in astrocyte-specific S100β (a putative marker of astrocyte distress) and found that while levels were increased by stress, it was not correlated with atrophication. We did, however, find that levels of S100β were inversely correlated with the overall density of astrocytes. Together, these results provide a significantly more elaborate picture of how chronic stress can disrupt the organization of the PFC.

POS-TUE-115

SPATIOTEMPORAL MAPPING OF OLIGODENDROGENESIS BY NEURAL PRECURSOR CELLS FOLLOWING CUPRIZONE-INDUCED DEMYELINATION

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Oligodendrocyte progenitor cells (OPCs) are considered the principal cell type responsible for the production of new oligodendrocytes during remyelination of the central nervous system (CNS). Recent studies have demonstrated that neural precursor cells (NPCs) residing in the adult subventricular zone also exhibit the capacity to generate new oligodendrocytes following experimental demyelination. **Purpose:** We aimed to determine the relative contribution of NPCs and OPCs to oligodendrogenesis following cuprizone-induced demyelination. **Methods:** Adult *NestinCreER^{T2}: Rosa26-lox-STOP-lox-eYFP* mice were administered tamoxifen (0.3g/kg/day) for 4 days by oral gavage, inducing the permanent expression of yellow fluorescent protein (YFP) in NPCs and their progeny. Mice were subsequently fed 0.2% cuprizone for 6 weeks followed by 6 weeks recovery on normal food to enable the analysis of remyelination. Expression of YFP and other cellular markers were examined immunohistochemically to determine the fate and migratory potential of NPCs in the remyelinating corpus callosum (CC). **Results:** Rostrocaudal analyses of CC in the cuprizone-challenged mice (n=2) demonstrated that approximately 60% of YFP$^+$ NPCs commit to an oligodendroglial fate. There was robust recruitment of NPC-derived oligodendroglial cells, with a 14-fold increase in YFP$^+$Sox10$^+$ cells compared to unchallenged mice (n=3). Most of these cells were mature oligodendrocytes (YFP$^+$CC1$^+$). Their density in the remyelinating CC was highest adjacent to the dorsolateral corner of SVZ and decreased proportionally with distance in the mediolateral axis whereas OPC-derived mature cells (YFP$^-$CC1$^+$) were distributed in a converse manner. **Conclusion:** NPC fate-mapping has defined regions of the remyelinating CC in which SVZ-derived precursors are the dominant progenitor cell type contributing to oligodendrogenesis. Our data reveal the important contribution of SVZ-derived NPCs during CNS remyelination.

POS-TUE-116

ALCOHOL AND SUCROSE SELF-ADMINISTRATION AFTER LOCAL CENTRAL RELAXIN-3/RXFP3 SIGNALLING DISRUPTION IN IP RATS

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Purpose: Alcoholism is a chronic relapsing disorder and a major cause of morbidity, accounting for 10% of disability-adjusted life years lost in industrialized countries. We have shown that central adminsitration of RXFP3 receptors reduces alcohol consumption and seeking (Ryan PJ et al, in revision). Thus, our working hypothesis is that ascending relaxin-3-containing networks regulate drug-seeking and local RXFP3 antagonism in key brain areas, such as the lateral hypothalamus, should recapitulate these effects. Methods: Rats were trained to self-administer ethanol (10%) on a FR3 ratio, with vanilla essence as an olfactory cue (S+) and a 1-s light stimulus as a visual cue (CS+) that indicated availability of ethanol. After stabilisation of ethanol responding, rats underwent stereotaxic surgery to position a cannula above the target brain loci. After recovery and re-stabilisation on ethanol, rats received injections via the cannula of vehicle or the single-chain RXFP3 antagonist, R3(B1-22)R (1-10 µg) into the lateral hypothalamus (LH) and were tested for self-administration. Results: In contrast to icv administration that reduced responding, pharmacological RXFP3 antagonism (R3(B1-22)R, 10 µg bilaterally) in the LH increased self-administration of alcohol ($F(5,25)=5.282$, $p<0.05$). Studies of sucrose self-administration are in progress to assess the specificity of the effects observed. Conclusion: These data suggest topographically distinct, functionally competing circuits are altered by relaxin-3 network activity. These studies further elucidate the sites and mechanisms by which RXFP3 signalling modulates alcohol-seeking in rats, and the broad neurochemistry of reward-seeking, with implications for therapy of alcoholism and addiction.

POS-TUE-117

AXONAL DELAY SELECTION BY SPIKE-TIMING-DEPENDENT PLASTICITY IN RECURRENT NETWORKS OF SPIKING NEURONS RECEIVING OSCILLATORY INPUTS

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Purpose: Understanding how learning rules, such as spiking-timing-dependent plasticity (STDP), change the structure of neural networks can help infer how the brain learns and processes information. STDP has been shown to selectively potentiate feedforward connections with specific axonal delays, enabling functions such as sound localization in the auditory brainstem of the barn owl. We investigate the selective potentiation by additive STDP of recurrent connections with a range of axonal delays with oscillatory input. **Methods:** Analysis and simulations of leaky integrate-and-fire neurons are used to study recurrent networks driven by oscillatory inputs and with a range of axonal delays between 1 and 10ms. **Results:** For input frequencies of 100, 120, 140, 180, 240, and 300Hz, simulations (in agreement with analysis) show that learning results in narrow axonal delay profiles with weighted means of 7.8±0.4ms, 6.4±0.4ms, 5.5±0.4ms, 4.1±0.6ms, 2.9±0.6ms, and 2.2±0.7ms, respectively. For frequencies up to 180Hz, this leads to the networks selectively showing stronger oscillatory responses to this training frequency; however, higher frequencies require faster neuronal and synaptic dynamics. This single network model is extended to axonal delay selection between multiple groups that receive out-of-phase, oscillatory inputs, where the network learns to become selective to both the training frequency and time lag between the inputs. **Conclusion:** These models can be applied to missing fundamental pitch perception in the auditory brainstem and the formation of neuronal ensembles (or cell assemblies) in the cortex.

POS-TUE-118

NOVEL KAPPA OPIOID RECEPTOR AGONIST 2-METHOXY-METHYL SALVINORIN B (MOMSAL B) ATTENUATES COCAINE SEEKING IN RATS

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Purpose: Activation of Kappa-opioid receptors (KOPr) by traditional arylacetamide agonists and the novel neoclerodane diterpene Salvinorin A (Sal A) atteunates cocaine-seeking behaviour in pre-clinical models of addiction. However, adverse-effects such as sedation, depression and aversion limit their clinical utility. The Sal A analogue, 2-methoxy-methyl salvinorin B (MOM Sal B) is a longer acting Sal A analogue with high affinity for KOPr. We hypothesise that novel KOPr agonists may be synthesised with desired anti-addiction effects and fewer side effects. **Methods:** In this study, rats were trained to self-administer cocaine, whereby, depression of an active leaver delivered a jugular infusion of cocaine (0.5 mg/kg/infusion, FR-5 schedule of reinforcement). MOM Sal B was tested for its ability to modulate cocaine-seeking behaviour in rats using the cocaine-primed induced reinstatement model. Spontaneous and cocaine induced locomotor activity and sucrose reinforcement were also measured to determine sedative effects and effects on natural reward. **Results:** MOM Sal B (0.3 mg/kg) successfully attenuated cocaine-prime induced drug-seeking in a KOPr dependent manner ($p<0.05$; n=5-6 per group). No change in motor activity was observed in either drug naïve or cocaine self-administrating rats (n=7). However, MOM Sal B significantly suppressed operant lever press responding for sucrose reinforcements ($P < 0.01$; n=5-7) suggesting a non-selective effect on reward. **Conclusions:** this study shows that the novel KOPr agonist, MOM Sal B, successfully attenuates cocaine-seeking behaviour in a pre-clinical model of addiction without causing sedation. This effect may be due to modulation of the natural reward pathway, as sucrose reward was also significantly reduced.

POS-TUE-119

INVESTIGATING NEUREXIN AND NEUROLIGIN FUNCTION USING DROSOPHILA MELANOGASTER

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Purpose: As the brain develops, huge numbers of neurons synapse with each other to form complex networks. Even after these initial connections are made, the circuitry of the brain is plastic and is altered to reflect experiences. Neurexins and neuroligins are cell adhesion molecules important for formation, modulation and activity-dependent specification of synapses. Their functional importance is underlined by the fact that mutations in these genes are associated with cognitive disorders in humans, including autism. *Drosophila melanogaster* is a useful system in which to study the function of these highly conserved genes *in vivo*. **Methods:** We are employing behavioural assays to assess function of neurexin and neuroligins, to relate abnormalities in synaptic communication to basic behaviours and complex cognitive tasks. **Results:** *neurexin1* knockouts (n=45) have disrupted circadian rhythm and more fragmented sleep (more, shorter sleep bouts: $p<0.05$) than control animals (n=131,16). Overexpression of *neurexin1* (n=30,32) points towards fewer, longer-lasting sleep bouts, however genetic controls show similar changes. Overexpression of *neuroligin2* (n=22,37) leads to more sleep bouts compared to wild-type (n=131, $p<0.05$); however this change is also seen in apparently leaky (supported by qPCR data) genetic control animals (UAS-nlg2/+: n=38, $p<0.05$). Similar genetic perturbations revealed no significant effects on grooming behaviour, though possible defects in motor coordination of *neurexin1* knockouts were noted. **Conclusion:** Sleep is thought to be important for synaptic plasticity and homeostasis. Disruption of the single neurexin gene in *Drosophila* alters sleep patterns. Changes caused by overexpression of a single neuroligin (of four) may be more subtle, possibly due to compensatory genetic mechanisms; follow up experiments using knock-down techniques are planned. We aim to further investigate involvement of these genes in plasticity processes (sleep, learning and memory), as well as basic motor function.

POS-TUE-120

REGIONALISED BIOCHEMICAL CHANGES IN THE BRAINS OF THE HUNTINGTON'S DISEASE R6/1 MOUSE MODEL

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Introduction: The Huntington's disease (HD) R6/1 mouse model recapitulates symptoms of the human form of the disease, including progressive cognitive and locomotor decline. Pathologically, HD primarily involves the loss of neurons from the striatum. The mechanisms involved in the specific loss of striatal neurons are unknown, but may involve pathological changes from other areas of the brain. This study analysed biochemical changes in different regions of the brain of the HD R6/1 mouse. **Methods:** HD R6/1 mice and wild-type littermates were culled at 17 weeks of age (n=8 per group) and four different regions of the brain were dissected out; frontal cortex, striatum, hippocampus and cerebellum. These brain regions were analysed for biochemical changes using SDS-PAGE and western blotting. **Results:** In the frontal cortex, levels of phosphorylated Akt, phosphorylated glycogen synthase kinase-3, phosphorylated JNK and phosphorylated tau were all increased ($p<0.05$). The striatum and hippocampus showed an increase in a 35kDa fragment of TDP-43 ($p<0.05$). In the cerebellum, levels of the glial glutamate transporter-1 (GLT-1) were decreased ($p<0.05$). **Discussion:** The changes observed in the frontal cortex suggest an upregulation of phosphorylated proteins in HD. Abnormal cleavage of TDP-43 may contribute to pathological changes occurring in the striatum and hippocampus, while in the cerebellum changes to GLT-1 suggest glial-mediated excitotoxicity has a role in HD. These preliminary data indicate the broad range of cognitive, psychiatric and locomotive deficits in HD may be the result of multiple biochemical abnormalities that occur in distinct regions of the brain.

POS-TUE-121

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

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Background: The Amyloid Precursor Protein (APP) of Alzheimers disease (AD) has been extensively studied as the source of the amyloid β protein (Aβ), a major pathological hallmark of AD. We have recently described proteolytic processing at the N-terminal region of APP resulting in the release of secreted APP N-terminal fragments (APP NTFs) (Vella et al FASEB J 2012). The metalloprotease, meprin β, has been identified as a protease responsible for cleaving APP to generate the APP NTFs. The production of the 17-28 kDa APP NTFs is developmentally regulated (Vella et al FASEB J 2012). To define the regulation of APP-NTFs we investigated their generation under different neuronal and cellular conditions. Specifically, we investigated what followed neuronal KCl depolarisation and altered cholesterol metabolism. **Methods:** Neuronal KCl depolarization: SH-SY5Y cells or mouse cortical neurons were depolarized by replacing the growth media with fresh media containing 100 mM KCl. After 5, 60 and 120 minutes of treatment, the media was collected for analysis. Neuronal cholesterol depletion: SH-SY5Y cells or mouse cortical neurons were depleted of cholesterol by replacing the growth media with fresh media containing 5 mM methyl-β-cyclodextrin. After 10 and 20 minutes of treatment, the media was collected for analysis. Western blotting was performed on the collected media and an NT specific APP antibody was used to detect APP NTFs. Quantitation was performed with Image J. Experiments were repeated at least 4 times and statistical differences analyzed with SPSS. **Results:** KCl depolarization treatment resulted in an increase in APP NTFs production at the 5 and 120 min time points in both SH-SY5Y and in primary neurons. An increase in secreted APP ectodomain also occurred. Cholesterol depletion caused a decrease in secreted APP and APP NTFs levels at the 10 and 20 min time points. An increase in secreted APP ectodomain was also measured. **Conclusions:** This data indicates that the generation of APP NTFs is modulated by both cellular depolarization and cholesterol depletion. The effects following depolarisation or cholesterol depletion on meprin-beta activity is being investigated.

POS-TUE-122

ELECTROPHYSIOLOGY OF NUCLEUS INCERTUS NEURONS: HETEROGENEOUS RESPONSES TO CRF AND COHERENCE WITH HIPPOCAMPAL THETA RHYTHM

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Purpose: Relaxin-3 (RLN3) is a neuropeptide highly expressed in the nucleus incertus (NI), a cluster of large neurons in mammalian hindbrain involved in an ascending arousal pathway. NI neurons express high levels corticotropin-releasing factor (CRF) type-1 receptor (CRF-R1) and are stress and CRF responsive; but the precise anatomical and physiological characteristics of NI/RLN3 neurons are unclear. Therefore the aims of this study were to characterize CRF interaction with NI neurons. **Methods**: Studies in adult, male Sprague-Dawley rats utilised immunohistochemistry, retrograde-tract tracing, in vivo extracellular unit recordings with juxtacellular labelling and in vitro patch clamp recordings in brain slices. **Results**: We identified a significant population of NI neurons containing CRF-R1, including all RLN3 neurons. Retrograde-tracing from NI revealed inputs from CRF neurons of the lateral preoptic hypothalamus. In vivo recordings in urethane-anesthetised rats revealed that while most NI cells excited by intracerebroventricular CRF infusion were RLN3-positive, all inhibited cells were RLN3-negative. In vitro recordings demonstrated that CRF activation of NI/RLN3 neurons was tetrodotoxin-insensitive and could be blocked by CRF-R1 antagonist, indicating a direct, postsynaptic action of CRF on CRF-R1. In vivo recordings revealed that RLN3-positive and negative neurons show coherent firing with hippocampal theta activity. **Conclusion**: Our data suggests the NI is a heterogeneous neuronal population and a hindbrain site of CRF action, consistent with a modulatory role in arousal and cognitive processes in response to neurogenic stressors.

POS-TUE-123

DYSFUNCTION IN SOCIAL COGNITION BUT SPARING OF SHORT-TERM SPATIAL MEMORY IN A MOUSE MODEL OF METHAMPHETAMINE-INDUCED PSYCHOSIS: CONTRASTING ROLES OF BDNF

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Purpose: Methamphetamine (METH) users have an increased prevalence of psychosis and schizophrenia, including cognitive and negative symptoms. Brain-derived neurotrophic factor (BDNF) is implicated in the pathophysiology of schizophrenia, as well as neuronal responses to stimulant drugs. However, the interaction of METH and BDNF in psychosis remains unclear. **Methods**: BDNF heterozygous mice (HETs) and wild-type (WT) littermates were treated with METH during young adulthood and tested in adulthood for short-term spatial memory in the Y-maze and social cognition in the Crawley 3-chamber social interaction paradigm. Arm visits in the Y-maze and chamber time/cup sniffing in the social interaction task were analyzed with Cleversys TopScan software. Group differences were analyzed with repeated-measures ANOVA and paired t-test. **Results**: Preliminary analysis (n=6-10 animals per treatment group) of Y-maze performance found no disruption of short-term memory spatial memory, with all groups showing a preference for the novel arm (p=0.001). In the sociability phase of the social interaction task, METH disrupted preference for the social chamber in WT mice, but not in BDNF HETs (p=0.02). In contrast, during the novelty preference phase when a new stranger mouse was introduced, METH-treated BDNF HETs showed selective disruption of their preference for sniffing the novel mouse over the familiar mouse (paired t-test). **Conclusions**: METH treatment in young-adulthood altered social behaviour in the 3-chamber paradigm. BDNF depletion can have opposite effects on METH-induced social impairment depending on the trial phase. In contrast, hippocampus-dependent short-term spatial memory appeared unaffected by METH treatment in either genotype. This 'two hit' model may add to our understanding of the role of BDNF in the development of negative symptoms, such as social withdrawal, in schizophrenia and METH-induced psychosis.

POS-TUE-124

EFFECT OF WESTERN DIET CONSUMPTION ON SPATIAL ALTERNATION AND BRAIN SEROTONIN MEASURES IN THE RAT

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Purpose: An increased prevalence of dementia and other cognitive disorders has been associated with the increased consumption of the western diet. This suggests there is a link between diet and cognitive function. This study investigated the differences in body weight, epididymal fat, basal locomotor activity and spontaneous alternation behaviour in the Y-maze in a western diet rat model of consumption. **Methods:** Male Long-Evans rats (n=12/group) were fed with either a western diet (21% fat content) or control diet (6% fat content) for 12 weeks. Rats underwent behavioural testing 12 weeks after diet commencement with basal locomotor activity assessed by Med Associates locomotor box and spontaneous alternation behaviour measured by the percentage of correct choices in the Y-maze test. Following this western blotting was also employed to analyse 5HT-2C expression in the striatum. **Results:** While the consumption of the western diet did not significantly increase final body weight (358g vs. 374g, control and western diet fed rats respectively, p=0.099) it did produce a significant increase in epididymal fat weight (p=0.0001). Behavioural analysis showed no difference in spatial alternation (p=0.33) and basal locomotor activity (p=0.36). However, an increase in 5HT-2C receptors in the striatum was observed (p=0.038). **Conclusion:** The consumption of a western diet in male Long-Evans rats for 12 weeks resulted in the increase of epididymal fat weight with an associated increased expression of 5HT-2C receptors in the striatum. These observed increases in epididymal fat and 5HT-2C receptors are not affected by body weight.

POS-TUE-125

A META-ANALYSIS OF BRAIN AREAS ALTERED IN DEPRESSED SUBJECTS

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Purpose: Depression continues to be a major burden to society with an estimated 350 million people globally suffering from depression. We sought to quantitatively summarise which brain areas are altered in depressed patients relative to healthy controls when performing either an emotional or cognitive task or resting-state connectivity using a published meta-analysis technique, activation likelihood estimation (ALE) (www.brainmap.org). Further we sought to compare and contrast the brain areas altered in the three conditions. **Methods:** We reviewed the literature from relevant databases. Only studies that contrasted depressed patients with controls, involved whole brain analysis, reported activation coordinates and utilised PET, SPECT, ASL or fMRI technology with a resting-state connectivity, emotional or cognitive processing condition were included. Using a threshold for false discovery of $p<0.05$ and the recommended cluster threshold, we performed ALE meta-analysis on the results of the resting-state (n=21), emotion (n=29) and cognitive processing (n=15) publications and then used the subtraction analysis to compare the meta-analyses. **Results:** Results identified seven, five and eight statistically significant clusters respectively, which were localised to brain areas using the Talairach Daemon template. Contrast analyses revealed three overlapping areas in the brain when comparing resting-state and cognitive meta-analyses or resting-state and emotion meta-analyses. There were no significant areas of overlap when contrasting cognitive and emotional meta-analyses. Significant differences were found between emotional and cognitive meta-analyses with left and right amygdala being more significantly altered for emotion compared to cognition. **Conclusion:** Whilst there is overlap in altered brain regions between task and resting-state conditions, this was not the case between the emotional and cognition task conditions.

POS-TUE-126

EXERCISE MODIFIES THE DEVELOPMENT OF DEPRESSION-RELATED BEHAVIOURS DURING ETHANOL WITHDRAWAL

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Purpose: Withdrawal from chronic alcohol consumption is associated with the emergence of behavioural changes such as greater anxiety, anhedonia and depression. These constitute a major challenge when treating patients with alcoholism-related disorders. There is continual interest in the development of non-drug therapeutic approaches. Methods: Female C57/bl6 mice (n = 12) underwent a 6-week paradigm of self-administration of alcohol (10% ethanol v/v). A control group comprised mice provided with normal water (n = 12). Ethanol was withdrawn for 2 weeks during which half the ethanol-drinking and water controls were provided access to a running-wheel. Mice were then tested on a variety of behavioural tests and serotonin receptor function was assessed using the selective 1A receptor agonist 8-OH-DPAT. Results: After 2-weeks of ethanol deprivation, mice displayed increased immobility time in the forced-swim test (FST) ($p < 0.05$), reduced saccharin preference ($p < 0.001$) and increased latency to feed in the novelty-suppressed feeding test (NSFT) ($p < 0.01$) compared to water-only controls. The provision of free-choice wheel-running during the withdrawal period attenuated the behavioural changes in the FST and NSFT. Withdrawal from ethanol resulted in 5-HT1A autoreceptor hypersensitivity measured by hypothermic response following 8-OH-DPAT administration, but this was not observed in mice provided with running-wheels. In contrast, 5-HT1A heteroreceptor function determined by 8-OH-DPAT-induced corticosterone release was unaffected. Conclusion: Our results reaffirm the impact of alcohol withdrawal on psychiatric behaviour, and demonstrate that non-pharmacological interventions such as exercise may be a feasible therapeutic strategy to adopt for the treatment of alcohol withdrawal symptoms. However, we have also identified a specific change in 5-HT1A receptor function that may be relevant for the future development of pharmacotherapies.

POS-TUE-127

REPEATED BOUT RTMS ON SPATIAL WORKING MEMORY: A COMPARATIVE OPEN-LABEL STUDY OF TWO CORTICAL AREAS

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Purpose: Working memory (WM) is the transient storage and processing of information. A significant component of WM is spatial working memory (SWM). Positively correlated with both fluid and general intelligence, SWM involves the temporary storage and manipulation of spatial information such remembering different visited locations, as well as where an object is relative to another object. Here we investigate the potential for rTMS to improve SWM after six sessions of rTMS treatment in healthy young people. **Methods:** Thirty healthy participants (10f; 20m) were randomly divided into three groups: rTMS of dorsolateral prefrontal cortex (DLFPFC), rTMS of posterior parietal cortex (PPC), and no rTMS control. Participants in the rTMS groups completed six rTMS sessions of 300 pulses per session (10 cycles of 30 pulses at 5 Hz, with 10 s intercycle rest) every 2nd day over 2 weeks duration. Pre and post SWM testing for all participants was completed using several subtests from the Cambridge Neuropsychological Test Automated Battery (CANTAB). **Results:** Following the rTMS intervention, no differences were observed in the duration required to complete testing. Significant differences were found in the repetitive searching patterns used and reduced errors between the rTMS groups and control (P=.027 and.021 respectively); however post-hoc tests did not show significant differences between the DLPFC and PPC, despite DLPFC demonstrating a moderate effect size (ES) in strategy and errors compared to PPC (ES=.49 and.73 respectively). **Conclusion:** Repeated bouts of rTMS facilitates SWM in young healthy people. With moderate ES differences, further study is required to elucidate differences observed rTMS in DLPFC and PPC using rTMS.

POS-TUE-128

A ROLE FOR THE MGLU5 RECEPTOR IN EXTINCTION OF COCAINE-SEEKING BEHAVIOUR

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Purpose: The mGlu5 receptor is known to be involved in learning and memory formation. Here we examined whether mGlu5 receptor signalling is important for extinction of drug-associated operant responding and context. **Method:** Rats were trained to lever press for cocaine (0.3mg/kg/infusion) and then assigned to one of three conditions. Group Lever Extinction (n = 31) were placed in the operant chambers and allowed to lever press under extinction conditions. Group Context Extinction (n = 12) were placed in the same chambers, but in the absence of the levers. Group Abstinence (n = 10) remained in their home cages. No further cocaine reinforcement was supplied. After each session, rats received either MTEP (2mg/kg i.p.) or vehicle. Following 9 days of extinction/abstinence, all rats were given a priming dose of either cocaine (10mg/kg i.p.) or saline. They were then placed in the operant chambers and allowed to lever press under extinction conditions. **Results:** MTEP had no effect on the decline in responding that occurred across extinction ($p >.05$). Responding was reliably reinstated by the cocaine prime ($p <.05$). Injections of MTEP during lever extinction and during abstinence had no effect on subsequent primed drug-seeking behaviour. In contrast, responding after a prime was higher in rats treated with MTEP compared to vehicle in group Context Extinction. **Conclusion:** These findings suggest that mGlu5 receptors are more important for the extinction of context-reward associations than extinction of the response-reward association. Further, this implies dissociation between the circuitry underlying context and lever extinction, with implications for developing more effective behavioural therapies for promoting abstinence and preventing relapse in drug-dependent individuals.

POS-TUE-129

THE FUNCTIONAL ROLE OF OREXIN/HYPOCRETIN NEUROPEPTIDES IN CONTEXT INDUCED REINSTATEMENT OF DRUG SEEKING

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Orexin/Hypocretin are neuropeptides involved in reward and addiction. Orexin neurons within the lateral hypothalamus (LH) are activated during context- induced reinstatement of drug seeking. Here, we used orexin antisense vivo morpholinos to study the specific role of the orexin neuropeptides in context-induced reinstatement of alcoholic beer-seeking. Rats were trained to respond for 4% (vol/vol) alcoholic beer in one context (Context A) followed by extinction in a second context (Context B). Rats were tested in the training context, A (ABA) or the extinction context, B (ABB). Return to the training context elicited reinstatement whereas return to the extinction context elicited low responding. Administration of orexin antisense morpholinos in LH in Experiment 1 produced effective and specific knockdown of orexin peptides without disrupting the expression of melanin concentrating hormone (MCH). We hypothesised that suppression of orexin peptides in the LH would reduce reinstatement. In Experiment 2, surprisingly, we found that knockdown of orexin peptides resulted in higher reinstatement compared to a control group with sense vivo morphilino. Administration of orexin antisense vivo morpholino in LH had no effect when tested in the extinction context (ABB). Further analyses examined the relationship between the specific site of orexin/hypocretin knockdown and magnitude of reinstatement as well the effect of the orexin antisense vivo morpholino on other proteins expressed by orexin/hypocretin neurons, especially prodynorphin.

POS-TUE-130

ALTERED MIRNA EXPRESSION IN THE DORSAL STRIATUM OF COCAINE-'RELAPSE' VULNERABLE ANIMALS

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Purpose: microRNA (miRNA) regulate the expression of genes through translational repression or transcript degradation. A dysregulated profile of miRNA expression has been linked with psychopathologies including addiction. We previously reported that cocaine-'relapse' vulnerability in rats is associated with down-regulation of synaptic plasticity associated genes in the striatum, including activity-regulated cytoskeletal protein (ARC) and dopamine and Group 1 metabotropic glutamate receptors. Here we aimed to identify miRNA potentially involved in relapse vulnerability through interactions with synaptic plasticity genes within the dorsal striatum (DS). **Methods:** miRNA microarray analysis was performed on RNA samples from the DS of animals previously phenotyped as cocaine-'relapse' vulnerable (n=6) or resistant (n=6). Agilent GeneSpring GX analysis software was used to identify candidate miRNAs that may regulate ARC and other addiction-associated genes. Confirmatory qPCR was performed on tissue derived from macrodissected subregions of the DS, including the dorsomedial (DM) and dorsolateral (DL) subregions. **Results:** Approximately 20% of DS miRNA were shown to be significantly altered using microarray analysis. Our analyses identified significantly altered miR-221 and miR-431 expression in the DMS. Additionally, miR-431 also showed significantly altered expression in the DLS. Furthermore, miR-212, shown to play a critical role in compulsive cocaine consumption, was increased in the DLS and decreased in the DMS. **Conclusion:** These results suggest that miR-221 and miR-431 may negatively regulate ARC expression in the DS and contribute to synaptic plasticity deficits commonly associated with addiction vulnerability. Interestingly, given the distinct roles ascribed to the DM and DL striatum in decision-making tasks, we identified a sub-region specific expression pattern for miR-212.

POS-TUE-131

A MODEL OF VIRAL ILLNESS IN PREGNANCY IN THE PRECOCIAL SPINY MOUSE: A POSSIBLE PRENATAL ORIGIN OF MENTAL ILLNESS

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Background: Considerable human and animal-based evidence supports an association between maternal illness in pregnancy and long-term adverse effects on the health of the offspring, including mental health conditions such as attention deficit disorder, autism, and schizophrenia. The effect of varying severities of an infection, in mid gestation, where fetal development continues through a long pregnancy, is investigated in this study. **Methods:** Pregnant spiny mice were injected with 0.5 mg/kg (low dose) or 5mg/kg (high dose) the viral mimetic, Poly I:C (double-stranded RNA that targets the Toll-like receptor-3) or saline at 20 days gestation (term is 39 days). The dams were either killed at 1 (n=5) or 24 h (n=5) post injection for collection of placentas and fetal tissues for genomic analysis, or were left to give birth naturally (n=12-15) and offspring behaviour were assessed at 20 to 40 days postnatal age using the open field test, novel object recognition test, elevated plus maze and pre-pulse inhibition test. **Results:** Open field testing of offspring showed that only the low dose of Poly I:C treatment during pregnancy significantly reduced activity in the open field. The novel object recognition test showed that offspring prenatally exposed to both doses of Poly I:C had diminished capacity to remember and recognise objects. Animals exposed to the high dose of Poly I:C showed a reduced pre-pulse inhibition indicating abnormalities in sensorimotor gating. **Conclusion:** This study shows varying severities of a viral illness have different impacts on the maternal placental and fetal innate immune system and the long-term behaviour of the offspring. This study provides evidence of the short-term changes in the intrauterine environment that result from a viral infection and the subsequent long-term affect on the neurodevelopment of the newborn.

POS-TUE-132

MIND BENDING BACTERIA: *WOLBACHIA,* A COMMON BACTERIAL SYMBIONT OF INSECTS, INFLUENCES BEHAVIOUR IN DROSOPHILA MELANOGASTER

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Purpose: *Drosophila melanogaster* has been used extensively as a model system to investigate the underlying genetic mechanisms that facilitate behaviour. *Drosophila* are commonly infected by *Wolbachia*, a bacterial symbiont that was recently shown to influence olfaction and locomotion. What impact *Wolbachia* has on more complex behaviors and the underlying mechanism by which these behaviours are modified were unclear. **Methods:** Two *Wolbachia* strains that establish low (*w*Mel) or extreme (*w*MelPop) bacterial densities in adult fly brains were compared to *Wolbachia* free counterparts in both genders, at three different time points (2, 5 and 8 days of age) and at two different temperatures (24°C and 29°C). Three behaviours were examined: visual attention (n = 360/condition), male aggression (n = 40/condition) and arousal threshold (n = 51/condition). qPCR was used to estimate changes in gene expression of four genes within the dopamanie biosynthetic pathway (*ppo-I*, *ppo-II*, *ple*, and *aph-4*; n = 5/condition). **Results:** *Wolbachia* was shown to increase visual attention in adult *Drosophila* under all conditions tested. *Wolbachia* increased arousal thresholds, thus adult *Drosophila* required greater external stimulation before a response was observed. Male aggression was decreased only in *w*MelPop infected flies. Changes to 2/4 dopamine biosynthetic pathway genes was observed, the greatest impact was associated with the *w*MelPop infection. **Conclusion:** Our work demonstrates for the first time that a bacterial symbiont can influence the biosynthesis of neurotransmitters and consequently complex behaviours in an animal host. The ability of *Wolbachia* to manipulate *Drosophila* behaviour provides an opportunity to explore the general neurological mechanisms that control behaviour.

POS-TUE-133

RXFP3 ANTAGONISM DECREASES ALCOHOL CONSUMPTION & ALCOHOL SEEKING IN RATS

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The neuropeptide, relaxin-3, is expressed predominantly in the hindbrain nucleus incertus and relaxin-3 neurons project widely to forebrain areas that express high levels of its cognate receptor, RXFP3, including amygdala, bed nucleus of the stria terminalis, hippocampus and hypothalamus. These anatomical data and findings that relaxin-3 can alter food intake, and interact with CRF systems, support the hypothesis that relaxin-3 may modulate drug seeking behaviour. **Purpose:** To investigate the effect of RXFP3 antagonism on alcohol consumption and seeking. **Methods:** Alcohol-preferring (iP) rats were trained to self-administer ethanol (10% v/v) or sucrose (0.7-1% w/v), then injected icv (intracerebroventricularly) with RXFP3 antagonist, R3(B1-22)R, prior to (1) self-administration or (2) cue-induced reinstatement following extinction. **Results:** R3(B1-22)R (3-30 µg) decreased self-administration of alcohol in a dose□related manner (1-way ANOVA, $F(4,70) = 10.28$, $p < 0.0001$) and attenuated cue□induced reinstatement following extinction (1-way RM ANOVA, $F(2,26) = 30.07$, $p < 0.0001$). By comparison, RXFP3 antagonism (10 µg, icv) produced no significant change in self-administration of sucrose, suggesting a selective effect for alcohol. Interestingly, RXFP3 antagonists decreased cue-induced reinstatement following extinction for sucrose and alcohol (1-way RM ANOVA, $F(2,30) = 13.96$, $p < 0.0001$), suggesting RXFP3 blockade may reduce reward-seeking behaviour more generally. RXFP3 antagonist treatment had no effect on general ingestive behaviour, activity, or cognition in the paradigms used. **Conclusion:** These data suggest relaxin□3/RXFP3 signalling regulates alcohol intake and relapse□like behaviour, adding to current knowledge of the brain chemistry of reward-seeking.

POS-TUE-134

BEHAVIOURAL CORRELATES OF GESTATIONAL LOW DOSE ETHANOL EXPOSURE IN ADULT MICE

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Purpose: Extensive research has shown the detrimental effects of alcohol consumption during pregnancy, encompassed under a broad umbrella term, Foetal Alcohol Spectrum Disorders. The consequences of low dose exposure are not easily detectable but have been associated with long-term impacts on behaviour. The aim of this study was to examine the effect of a low dose ethanol during early gestation in mice on adult cognitive performance. **Methods:** Adult female C57Bl/6J mice mated and exposed to either 10% Ethanol or water for the first 8 days of pregnancy, and then water. Adult offspring were tested for spatial and working memory in a water maze (n=50), and attentional processing (n=32). Mice also underwent a broad behavioural screen to assess measures of locomotion, exploration, anxiety and depression. Hippocampal tissue was assessed by qPCR for markers of glutamate signalling. **Results:** EtOH exposed mice displayed hyperlocomotion ($p = 0.02$), increased exploration ($p = 0.037$), significant improvement in spatial memory ($p<0.05$), but no effect on working memory or attentional processing. Data showed altered motivation on the sucrose preference test and shorter latency to retrieve reward in the operant chambers. EtOH exposed mice had significant increase in VGLUT2, a marker of glutamatergic neurons. **Conclusions:** This novel study evaluated the cognitive phenotype of mice from a brief, low exposure to ethanol during early pregnancy. The main behavioural findings resemble hyperactivity and indicate improved performance. These changes were associated with an upregulation of VGLUT2 in hippocampal tissue indicating alterations in glutamate signalling. Taken together, these data suggest that low dose ethanol exposure during early gestation induces lasting behavioural and molecular changes.

POS-TUE-135

NEUROBIOLOGICAL CHANGES IN THE HIPPOCAMPUS FOLLOWING CHRONIC METHAMPHETAMINE ADMINISTRATION: A PROTEOMIC APPROACH

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Purpose: Methamphetamine is an addictive drug associated with severe psychopathology following chronic use. Methamphetamine is known to act on dopamine, serotonin and noradrenaline brain systems and previous studies have highlighted neurobiological changes in the striatum and amygdala following repeated administration. The hippocampus is also innervated by these neurotransmitters, however its role in methamphetamine abuse has not been determined. Therefore, this study assessed protein changes in the hippocampus following chronic administration. It was hypothesised that proteomic analysis would demonstrate evidence of neuroplasticity, specifically cytoskeletal and synaptic alterations, and neurotoxicity, particularly oxidative stress, in methamphetamine-treated rats. **Method:** Using methamphetamine locomotor sensitization as an animal model of methamphetamine abuse and psychosis, male Sprague Dawley rats (n=12) were randomly assigned to methamphetamine or saline groups. Following seven days of treatment and fourteen days stabilisation, both groups received a methamphetamine challenge injection (1ml/kg, i.p.) before hippocampi were rapidly dissected for protein analysis. Label-free shotgun proteomic analysis using mass spectrometry was used to detect differentially expressed proteins indicative of biological changes. **Results:** Methamphetamine administration produced significant changes in the hippocampal proteome. Biological triplicate analysis revealed 1030 reproducibly identified proteins in treated rats, and 964 in control rats, (protein FDR <0.27%). A number of differentially expressed proteins indicative of synaptic plasticity, such as GTPase KRas, and cytoskeletal alterations, such as vimentin, were found. Differentially expressed proteins indicative of oxidative stress, for example nitric oxide synthase, were also found. **Conclusion:** The hippocampal proteome produced by chronic methamphetamine administration identifies neurobiological mechanisms that may underpin learning and memory deficits, relapse and induced psychosis in chronic methamphetamine users.

POS-TUE-136

THE GALANIN-3 RECEPTOR (GALR3) ANTAGONIST, SNAP 37889, REDUCES ETHANOL CONSUMPTION IN ALCOHOL-PREFERRING MICE

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Purpose: Our laboratory has previously shown that the GALR3 antagonist, SNAP 37889, reduces ethanol self-administration in alcohol-preferring rats. The aim of the present study was to investigate whether this effect extends to mice in a 'binge drinking' model in this species. **Methods:** The Scheduled High Alcohol Consumption procedure (Rhodes et al., 2005) was adapted to induce binge drinking in C57BL/6J mice. Mice were exposed to 10% ethanol, 3 hours into the dark cycle, every third day for 4 hours; with no access to water during this time. Following 4 weeks of stable drinking patterns, mice were divided into groups (n=8) to receive either SNAP 37889 (10, 30, 80 -mg/kg, i.p), naltrexone (1.25 mg/kg, i.p), or vehicle. Ethanol intake was recorded initially an hour after treatment and then every hour for four hours. **Results:** A two-way ANOVA revealed a significant reduction in ethanol consumption for mice treated with 30 mg/kg of SNAP 37889, one hour post treatment compared to those treated with vehicle ($p<0.01$). No major reduction in alcohol intake was seen at the 10 or 80 mg/kg doses of SNAP 37889. Naltrexone (1.25 mg/kg i.p) significantly reduced ethanol consumption, compared to vehicle ($p<0.01$). **Conclusions:** Antagonism of GALR3 can significantly reduce alcohol consumption in a mouse model of binge drinking. RHODES, J. S., BEST, K., BELKNAP, J. K., FINN, D. A. & CRABBE, J. C. 2005. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiology & Behavior, 84, 53-63.

POS-TUE-137

MODELLING THE EFFECTS OF PATERNAL LIFESTYLE ON THE MENTAL HEALTH OF OFFSPRING

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Purpose: The influence of maternal stress on the *in utero* and postnatal development of offspring is well-established. By comparison, the extent of paternal influence is uncertain. Further investigation is required to identify and understand the mechanisms of paternal transgenerational transmission of behavioural and physiological traits. **Methods:** Stress constitutes a negative environmental stressor through fluctuations in the hormone cortisol (corticosterone in rodents). Male mice were treated with 4-weeks of corticosterone administered via drinking water after which they were mated with untreated females. Control mice received untreated water. Dams were allowed to litter down and the offspring underwent behavioural testing as follows. At PND3 and 8 weeks of age, anxiety and depression-related behaviours were examined through analysis of early ultrasonic vocalisations, elevated plus maze and the forced swimming test. Their physiological response to stress was determined by quantification of serum corticosterone levels at baseline and immediately after forced-swim stress. **Results:** Corticosterone-supplemented sires had shrinkage of adrenals (n=10; p<0.001) and a 65% reduction of corticosterone response to forced-swim stress. Their male offspring (n=11) had altered vocalisation patterns at PND3. Adult offspring spent less time in the open arm of the elevated plus maze (n=10-19; p<0.001). Adult female offspring spent less time immobile during a forced swim test (n=19; p<0.01). **Conclusion:** Exposure of the paternal lineage to chronic periods of elevated corticosterone is associated with the emergence of anxiety and depression-related behaviours in the offspring. This is a demonstration that environmental factors influence behavioural phenotypes across generations. Future work is required to determine the mechanisms underlying the transgenerational effects.

POS-TUE-138

ICV INJECTION OF RXFP3 ANTAGONIST BLOCKS PALATABLE FOOD CONSUMPTION IN MICE

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Purpose: Relaxin-3 is a newly identified neuropeptide with putative roles in arousal, feeding, motivation and reward drive. To explore these roles, we examined the effects of pharmacological activation and inhibition of the relaxin-3 receptor (RXFP3) in mice. **Methods:** Adult male C57B/6J mice were surgically implanted with indwelling ICV cannula and tested in feeding-related behavioural paradigms following injection of either selective RXFP3-agonist, RXFP3-antagonist, or vehicle. **Results:** Importantly, RXFP3-antagonist treated (4 nmol) mice consumed roughly half the amount of palatable food as vehicle controls (n=14,16, p<0.05). This effect appeared 'specific', as similar effects were not observed in relaxin-3 knockout mice (n=13,15, p=0.98), and activity in locomotor cells was unaltered (n=10,19, p=0.53). Due to the repeated exposure to palatable food during the training phase, this reduced consumption may be partly due to a reduction of food anticipatory activity (FAA), as in a separate cohort of food restricted mice RXFP3-antagonist treatment (0.75 nmol) reduced climbing behaviour (a measure of FAA) by half, compared to vehicle controls (n=9,9, p<0.01). In contrast, RXFP3-agonist treatment (1 nmol) had no effect on the consumption of palatable food (n=10,20, p=0.76) or regular chow (n=13,18, p=0.28), which is surprising considering the potent orexigenic effects following central infusion of RXFP3 agonists in rats, possibly eluding to important species differences in relaxin-3/RXFP3 systems. **Conclusion:** These studies demonstrate that endogenous relaxin-3 promotes palatable food consumption and FAA in mice – hence highlighting the potential of relaxin-3/RXFP3 as a promising target for the treatment of neuropsychiatric disorders that are associated with disregulated motivation and reward drive.

POS-TUE-139

THE RETROSPLENIAL CORTEX IN BEHAVIORAL VARIANT FRONTOTEMPORAL DEMENTIA AND ALZHEIMER'S DISEASE

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Background: The retrosplenial cortex (RSC) in the posterior cingulate gyrus has been implicated in spatial navigation and memory and is affected as early as the hippocampus (HC) in Alzheimer's disease (AD). While the HC has similar levels of atrophy in AD and behavioral variant frontotemporal dementia (bvFTD), memory deficits occur in only a subset of patients with bvFTD. There have been no studies assessing structural changes in the RSC in bvFTD. **Methods:** MRI scans from consenting patients with bvFTD (n= 15) and AD (n= 15), as well as age and sex matched controls (n=15), assessed at the Frontier clinic at Neuroscience Research Australia were evaluated. A manual tracing method was used to calculate regional brain volumes. Following institutional approvals, RSC tissue samples from patients with pathologically confirmed FTD (n=17), AD (n= 16) and controls (n= 19) from the Sydney Brain Bank were prepared for histological examination of neurons (using cresyl violet) and inclusion pathologies (using immunohistochemistry). The density of RSC neurons was determined using a modified optical dissector technique. ANOVA with posthoc Bonferoni tests were used. **Results:** RSC atrophy was confirmed in AD (p<0.001) and there is a significant 40% reduction in neuronal density in AD (p<0.01). No RSC atrophy (p>0.1) or neuronal loss (p>0.1) was found in bvFTD patients compared with controls. **Conclusion:** This study suggests that memory deficits in bvFTD do not relate to RSC neurodegeneration, whereas the amnesic syndrome associated with AD does. It also provides a possible explanation for the commonly seen spatial disorientation in AD but not bvFTD, indicating that performance on spatial orientation is contingent upon RSC preservation.

POS-TUE-140

DIFFERENTIAL EFFECT OF DOPAMINE D2 RECEPTOR BLOCKADE ON SCHIZOPHRENIA-LIKE DISRUPTIONS OF SENSORY GATING BY PHENCYCLIDINE, AMPHETAMINE OR APOMORPHINE

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Purpose: Schizophrenia patients have deficient P50 sensory gating, an information processing mechanism that occurs in response to repetitive auditory stimuli. P50 gating can be assessed by recording the electroencephalography (EEG) response to pairs of sound stimuli. Healthy subjects have a diminished response to the second sound, however schizophrenia patients have similar responses to both stimuli. Previous studies have shown that N40 gating, the rat equivalent of P50 sensory gating, can be disrupted by dopaminergic, glutamatergic and serotonergic mechanisms. However, their point of convergence is unknown. This study investigated if N40 sensory gating deficits caused by the glutamate NMDA receptor antagonist, phencyclidine, the dopamine releaser, amphetamine or the dopamine receptor agonist, apomorphine, can be ameliorated by the dopamine-D2 receptor antagonist drug, haloperidol. **Methods:** Male Sprague-Dawley rats (n=9-10/group) were surgically implanted with cortical surface electrodes. Test sessions comprised of 150 presentations of two 85 dB bursts of white noise, 500 ms apart (S1 and S2). **Results:** Saline-treated animals suppressed their EEG response to S2 to approximately 40% of the response to S1, indicating normal N40 sensory gating. Treatment with 2.5 mg/kg phencyclidine, 0.5 mg/kg amphetamine and 0.1 mg/kg apomorphine caused increased sensory gating ratios (S2/S1), indicating disruption of N40 gating. Pretreatment with haloperidol (0.25 mg/kg) prevented the disruptions caused by amphetamine and apomorphine but had no effect on phencyclidine-induced disruptions. **Conclusions:** These results indicate that, in contrast to amphetamine and apomorphine, phencyclidine caused a schizophrenia-like disruption of N40 sensory gating by a mechanism independent of dopamine D2 receptors. These results increase our understanding of the possible mechanisms behind sensory gating deficits in schizophrenia.

POS-TUE-141

A NOVEL TASK TO ASSESS COGNITIVE SYMPTOMS RELEVANT TO SCHIZOPHRENIA IN RODENTS

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Purpose: Cognitive symptoms of schizophrenia are debilitating and largely untreated. Translatable cognitive tasks used in both rodents and humans are required to improve treatments and evaluate animal models. This study aims to develop a novel task based on the widely used human continuous performance task to assess cognitive symptoms relevant to schizophrenia in rodents. **Methods:** Food restricted male Sprague Dawley rats (n=16) were trained in operant chambers on a novel signal detection task. Key features of the current task include short training procedure, self-paced initiation of trials, controlled subject placement during stimulus presentation and simultaneous delivery of reward with response. Signal manipulations determined the detection limits when the stimulus strength was reduced. This was followed by a test of reversal learning, and finally attention was taxed using distractors. **Results:** Rats were able to acquire the detection task; however they were not able to discriminate between variable signal strengths. During reversal rats readily extinguished the redundant pairing and acquired the new task. Omissions were extremely rare, indicating rats were attending to the stimulus and responding quickly. Impaired performance during distraction ($P<0.05$) differed for visual and auditory stimuli. **Conclusions:** Using a novel signal detection task we showed that rats were capable of responding with a high degree of accuracy at a fast pace, maintained attention for the duration of the session and demonstrated reversal learning. Distractor modality differentially altered measures of attention. These findings suggest the task has potential as a rodent analogue of the human continuous performance task. Further validation is required including an investigation of the neural circuitry involved.

POS-TUE-142

GABAERGIC MRNA EXPRESSION IS DIFFERENTIALLY REGULATED IN SUBREGIONS OF THE PREFRONTAL CORTEX IN RATS SENSITIZED TO METHAMPHETAMINE

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Purpose: GABAergic neurotransmission plays an important role in the regulation of the prefrontal cortex (PFC), with increasing evidence suggesting that dysfunctional inhibitory control of the PFC may underlie executive deficits in psychotic disorders. Methamphetamine is a psychostimulant that produces a progressive increase in locomotor response to drug administration (sensitization) that is believed to induce behavioural and neurobiological changes consistent with psychotic disorders. The aim of the present study was to investigate changes to GABAergic mRNA expression in subregions of the PFC following behavioural sensitization to chronic methamphetamine administration.**Methods:** Male Sprague Dawley rats (n = 12) underwent repeated methamphetamine (1ml/kg intraperitoneal (i.p.) days 1 & 7; 5mg/kg i.p. days 2 – 6) or saline (1ml/kg i.p.) injections for 7 days. Following 14 days of withdrawal, rats were challenged with acute methamphetamine (1mg/kg i.p.). Sixty minutes after drug challenge, brains were removed and the infralimbic (IF), prelimbic (PRL) and orbitofrontal (OFC) cortices were dissected out for quantitative PCR (qPCR).**Results:** Methamphetamine challenge resulted in significant sensitized locomotor response in methamphetamine pre-treated animals when compared to saline controls. QPCR revealed that metabotropic $GABA_B2$ mRNA expression was significantly upregulated in the IF while GAD_{67}, GAT-1 and GAT-3 mRNA expression were significantly upregulated in the PRL. Ionotropic $GABA_A$ receptor subunits α1, α3, α5 and β2 were significantly upregulated in the OFC. **Conclusion:** Alterations to GABA neurotransmission following chronic methamphetamine exposure are biologically dissociated between subregions of the PFC. These findings suggest that impaired inhibitory control of localised regions of the PFC may differentially regulate the cognitive and behavioural dysfunction commonly seen in mental illness.

POS-TUE-143

SURFACE LAPLACIAN OF CENTRAL SCALP ELECTRICAL SIGNALS IS INSENSITIVE TO MUSCLE CONTAMINATION

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Purpose: Electroencephalogram (EEG) in scalp electrical recordings is contaminated by persistent electromyographic activity (EMG). Here we investigated the effects of surface Laplacian processing on gross as well as persistent EMG contamination of EEG signals in electrical scalp recordings using awake, curare-paralysed, ventilated subjects to provide signals not contaminated with EMG. **Methods:** We made scalp electrical recordings on 6 subjects during passive and active tasks, on awake subjects in the absence and in the presence of complete neuromuscular blockade (cisatracurium plus respiratory support). Surface Laplacian estimators were compared to left ear and common average reference (CAR). Contamination was quantified by comparing power after paralysis (brain signal, B) with power before paralysis (brain plus muscle signal, B+M). Brain:Muscle (B:M) ratios for the methods were calculated using B and differences in power after paralysis to represent muscle (M). **Results:** Using surface Laplacian transforms, power differences due to paralysis were very small and not significantly different from zero (B:M ratio >6) in central and peri-central scalp leads. **Conclusions:** Scalp surface Laplacian transforms reduce muscle power in central scalp leads to (conservatively) less than one sixth of the brain signal, enabling the most reliable evaluation of brain signals currently available without paralysis. **Significance:** The method provides robust estimates for detecting high frequency (gamma) activity, for assessing electrophysiological correlates of cognition and disease, and also for providing a measure of brain electrical activity for use as a standard in the development of brain/muscle signal separation methods.

POS-TUE-144

DOES SURGICALLY-INDUCED INFLAMMATION WORSEN POSTOPERATIVE COGNITIVE OUTCOME AFTER ISOFLURANE ANAESTHESIA?

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Purpose: While the pathogenesis of post-operative cognitive dysfunction remains unclear, studies in young and aged animals suggest that anaesthesia and/or surgically-induced inflammation can affect cognitive outcome. We used a rat model to investigate the role of isoflurane anaesthesia alone or in the presence of surgically-induced inflammation. **Methods:** Male Sprague Dawley rats were subjected to isoflurane (n=9, 4h, 1.8% in 100% O_2). Controls were subjected to 10 min of O_2 (n=14). Laparotomy was performed in another group of isoflurane-treated animals (n=9), and the wound left open for 10min then sutured. Eight days after isoflurane exposure, cognition was tested in a fear conditioning paradigm. Rats were placed in a chamber in which they received a foot shock (1mA, 1s duration). When returned to the chamber the percentage of time spent in freezing behaviour was recorded as a measure of memory for the shock previously experienced in that chamber. One day after fear conditioning, rats were deeply anaesthetised and transcardially perfused. Hippocampal tissue was removed and processed for cytokine analysis (Bio-Plex™). **Results:** Rats exposed to isoflurane showed significantly decreased freezing behaviour compared to no-anaesthesia controls, indicating a memory impairment (25.4±9.4% vs 66.21±8.9%, $P<0.01$). Rats in the isoflurane plus surgery group also had impaired memory (35.3±7.5%) but this was not worse than isoflurane alone ($P>0.05$). Isoflurane exposure was associated with increases in pro-inflammatory cytokines in the hippocampus including IL-6 and TNF-α ($P<0.05$) compared with controls and isoflurane plus surgery significantly increased TNF-α in the hippocampus ($P<0.05$). **Conclusion:** The finding suggests memory is impaired following isoflurane, while added surgical trauma does not worsen memory. Memory impairment may be related to increased inflammatory cytokines in the hippocampus.

POS-TUE-145

LONG TERM EFFECTS OF OLANZAPINE AND BETAHISTINE ON SEROTONIN 5-HT$_{2A}$ RECEPTOR BINDING IN THE RAT BRAIN

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Olanzapine is widely used in treating multiple domains of schizophrenia symptoms through its binding profiles to various neurotransmitter receptors including 5-HT$_{2A}$ receptors (5-HT$_{2A}$R). Our previous studies have shown that 2 weeks co-treatment of betahistine (a H_1R agonist and H_3R antagonist) could reduce obesity induced by olanzapine. This study aimed to investigate whether long term co-treatment of olanzapine and betahistine affects 5-HT$_{2A}$R bindings. **Methods**: Female Sprague-Dawley rats were administered under 4 conditions (n=12): (1) Rats were treated with vehicle (control) during whole experimental period; (2) Co-treatment group (O+B): 5 weeks olanzapine treatment (1 mg/kg, t.i.d.), followed by 6 weeks co-administration of olanzapine with betahistine (9.6 mg/kg, t.i.d.); (3) olanzapine only (1 mg/kg, t.i.d.) treatment during weeks 7-11; (4) betahistine only (9.6 mg/kg, t.i.d.) treatment during weeks 7-11. Density of 5-HT$_{2A}$R were measured using [^{3}H]ketanserin. **Results**: Compared to the controls, olanzapine significantly decreased 5-HT$_{2A}$R bindings in accumbens shell and substantia nigra (SN) ($p<0.001$), as well as prefrontal cortex and cingulated cortex ($p<0.05$). Similar binding density changes in these nuclei were also observed in the O+B co-treatment group. However, betahistine reduced 5-HT$_{2A}$R bindings only in SN ($p<0.001$). **Conclusion**: Co-treatment of olanzapine and betahistine had similar effects as sole olanzapine treatment on 5-HT$_{2A}$R binding. These results suggest betahistine co-treatment would be a suitable combination therapy to reduce olanzapine-induced weight gain side-effects without affecting 5-HT$_{2A}$ receptors in the brain regions involved in treating schizophrenia symptoms.

POS-TUE-146

INDUCTION OF NRF2-REGULATED ANTIOXIDANTS IN CULTURED ASTROCYTES BY THE NEUROPROTECTIVE COPPER-BIS(THIOSEMICARBAZONATO) COMPLEX, CUII(ATSM)

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Purpose: We have demonstrated that the copper-bis(thiosemicarbazonato) complex, Cu^{II}(atsm), significantly attenuates disease symptoms in multiple animal models of Parkinson's disease and amyotrophic lateral sclerosis. This study seeks to elucidate the mechanisms by which Cu^{II}(atsm) elicits these effects. As oxidative damage and astrocyte activation were attenuated by Cu^{II}(atsm), potential stimulation of the neuroprotective antioxidant systems of astrocytes was investigated in vitro. **Methods:** Primary astrocytes cultured from mouse brains were treated with Cu^{II}(atsm) for 24h ($n\geq3$). Activation of signalling kinases was determined by Western blot to confirm the biological activity of Cu^{II}(atsm), which we have shown is accompanied by such activation. Induction of the predominantly glial transcription factor Nrf2, responsible for regulating antioxidant enzymes, was assessed by transfection of an antioxidant response element-GFP reporter. Upregulation of the Nrf2 targets heme oxygenase-1 (HO-1) and glutamate-cysteine ligase (GCL) was determined by Western blot and activity assay, respectively. As GCL controls the synthesis of the critical antioxidant glutathione, glutathione content and export were also determined. Primary cortical neurons were treated with media from Cu^{II}(atsm)-treated astrocytes to determine the influence of astrocyte-derived glutathione on neuronal glutathione content. **Results:** Cu^{II}(atsm) induced phosphorylation of kinases including Akt and ERK, and activation of Nrf2. Accordingly, Cu^{II}(atsm) treatment upregulated HO-1 and GCL, the latter enhancing glutathione content and elevating export of glutathione from astrocytes. Media from Cu^{II}(atsm)-treated astrocytes increased neuronal glutathione content. **Conclusions:** These results demonstrate that Cu^{II}(atsm) activates the transcription factor Nrf2 and upregulates astrocyte antioxidants. This action may contribute to the neuroprotective and disease-attenuating activity of Cu^{II}(atsm) observed in vivo, and indicates that Nrf2 may be a valuable therapeutic target for the treatment of neurodegenerative diseases.

POS-TUE-147

THE EFFECTS OF AGE ON THE RELATIONSHIP BETWEEN THE ELECTRORETINOGRAM, BLOOD FLOW AND VITREAL OXYGEN TENSION IN RATS

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Purpose: To quantify the relationship between the electroretinogram (ERG), ocular blood flow (OBF) and vitreal oxygen tension (pO2) during intraocular pressure (IOP) elevation in 2 and 14 month old rats. **Methods:** 2 (n=14) and 14 month (n=16) old Long Evans rats underwent 1 hour of a stepwise IOP elevation (10 to 120mmHg), achieved by adjusting the height of a Hanks reservoir attached to an anterior chamber cannula. This was followed by two hours of recovery. Throughout the protocol photopic ERGs (background: 15cd/m^2 stimulus: 2.03 log cd·ms^{-2}) OBF and pO2 (combined laser Doppler/fibre-optic oxygen sensor, Oxford Optronix) were continuously assayed. All data were expressed relative to baseline (%, 95% CI). **Results:** ERG decline with IOP elevation was more closely related to reductions in oxygen tension than blood flow in both young and older rat eyes. At moderate and low levels of ocular perfusion pressure (OPP = blood pressure – IOP), middle-aged rats show relatively higher levels of OBF (LDF(2-14mth): 58% better, $p<0.05$) compared to younger animals. Despite higher blood flow, there was lower vitreal oxygen tension at low OPPs in older eyes (pO2(2-14mth): 66.% better $p<0.05$). Following return of IOP to baseline, older eyes showed faster blood flow recovery [half recovery, 14mth: 0.13 vs 2mth: 1.03 min,*$p<0.05$] compared with younger eyes. Despite this old rats showed similar pO2 recovery [14mth: 119%(103,137) vs 2mth: 134%(117,150), $p=0.05$] and incomplete ERG recovery [14mth: 87%(75,98) vs 2mth: 105%(90,120), $p<0.05$]. **Conclusions:** Older eyes use more oxygen in order to maintain the same level of function and show poor recovery from stress, despite faster blood flow recovery.

POS-TUE-148

REDUCED PP-2A ACTIVITY AND TAU HYPERPHOSPHORYLATION: A TARGET FOR NOVEL ANTI-EPILEPTOGENIC THERAPIES?

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Aim : To investigate the role of PP-2A and tau hyperphosphorylation in the amygdala kindling rat model of temporal lobe epilepsy. **Background:** Protein phosphatase-2A (PP-2A) plays a role in neurodegenerative disease. One role of PP-2A is to dephosphorylate tau. Hypophosphorylated tau has been implicated in the pathogenesis of acquired forms of epilepsy. **Method**: Activity of PP-2A in relevant brain regions was assayed with a immunoprecipitation phosphatase assay kit, and the expression levels of PP-2Acatalytic subunit(PP-2Ac), PP-2A regulatory subunit B(PR 55), total tau and phosphorylation of tau on Ser 198 and 262 was measured with Western blotting. The effect of enhancing PP-2A activity in-vivo, by chronically treating with sodium selenate, during electrical amygdala kindling was compared with saline treatment. **Results**: PP-2A activities and PR55 expression were significantly decreased, and phosphorylation of tau on Ser 198 and 262 were both increased in amygdala, hippocampus and cortex of amygdala kindling rats (n=12). Furthermore, rats chronically treated with the PP-2A activator, sodium selenate had significantly slower progression of kindling (n=12). On molecular analysis the selenate treated kindled rats had significantly greater the PP-2A activities and PR55 expression, and decreased phosphorylation of tau, in amygdala, hippocampus and cortex, compared with rats treated with saline during the period of kindling. **Conclusion**: Amygdala kindling epileptogenesis is associated with a down-regulation of PP-2A activity, decreased expression of the PR 55 and an increase in tau phosphorylation, and pharmacologically enhancing PP2A activity with sodium selenate is anti-epileptogenic.

POS-TUE-149

CHANGES TO TDP43 EXPRESSION IN AGEING NEUROFILAMENT LIGHT PROTEIN KNOCKOUT MICE

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Purpose: The transactive response DNA-binding protein 43 (TDP-43) has been identified as a neurofilament light (NF-L) mRNA binding protein, which can stabilize neurofilament mRNA. Abnormal increased levels of TDP-43 are detected in the majority of patients with amyotrophic lateral sclerosis (ALS). Furthermore, a reduction of NF-L mRNA has been demonstrated in ALS. In this study, we investigated whether the deficiency of NF-L protein can result in alterations in TDP-43 localisation or expression associated with ageing. Methods: We studied protein levels of TDP-43 in different regions of brain and spinal cord (cortex, hippocampus, corpus callosum, cervical spinal cord, thoracic spinal cord and lumbar spinal cord) of aged 12 month old NF-L knockout (KO) mice (n=3) and wild-type (WT) control mice (C57BL/6) (n=3). Antibodies against TDP-43 and phosphorylated-TDP-43 were used for quantitative Western blot analysis. Results: There was a significant increase of TDP-43 protein levels in all studied regions of the brain and spinal cord in NF-L KO mice, as compared to WT mice. In addition, our preliminary data indicates an increase of phosphorylated-TDP43—particularly in the hippocampal region of NFL KO mice. Conclusion: Our findings suggest that NF-L protein or mRNA is a negative regulator for the expression of TDP-43 in the central nervous system and that absence of NF-L results in increased expression of TDP43. Future studies will determine if increased TDP-43 expression is associated abnormal TDP-43 phosphorylation and localization to the cytoplasm as occurs in human patients with ALS. Keywords: transactive response DNA-binding protein 43 (TDP-43), neurofilament-light (NF-L), amyotrophic lateral sclerosis (ALS)

POS-TUE-150

PROBDNF INDUCES APOPTOSIS AND INHIBITS PROLIFERATION AND MIGRATION OF OLIGODENDROGLIA

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In contrast to mature Brain-Derived Neurotrophic Factor (BDNF), proBDNF inhibits proliferation, migration, and neurite outgrowth of neurons and induces cell apoptosis via the signal pathway of p75NTR and sortilin. However, the effects of proBDNF on oligodendrocytes (OLs) are still unclear. Here, we showed that p75NTR, sortilin and proBDNF are expressed in cultured OLN-93 oligodendrocyte cells and analysed the functions of proBDNF in OLN-93 cells by MTT method for cell viability assay, BrdU staining for cell proliferation assay, scratch assay for cell migration observation and activated caspase 3 immunocytochemistry for cell apoptosis assay. The results indicated that proBDNF inhibited OLs proliferation and migration, decreased cell viability and promoted cell apoptosis; while anti-proBDNF neutralized the inhibition of proBDNF and promoted the OLs activities. However, these effects failed to be observed in the presence of p75NTRecd-FC and antibody of p75NTR, indicating that proBDNF induces the inhibitory effects on OLs via the p75NTR pathway. Moreover, immunohistochemistry in spinal cord injured rats showed that animals treated by proBDNF anti-serum had more proliferating OLs in lesion site and better functional recovery. These findings suggest that proBDNF is a detrimental factor after spinal cord injury.

POS-TUE-151

ABNORMAL SECRETION OF α-SYNUCLEIN IN PARKINSON'S DISEASE

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Purpose: While the cytoplasmic accumulation of misfolded alpha synuclein (α-syn) in neurons is implicated in the pathogenesis of Parkinson's Disease (PD), neither the native or pathological roles are fully understood. Active secretion of α-syn was recently demonstrated, with the protein identified in biological fluids of rodents and patients, and conditioned medium from cell lines. The dominant pathway for α-syn secretion remains unknown, in part because previous studies used transient transfection and extremely high levels of α-syn. In this study, we focussed on the role of the endosome lysosome system (ELS), an integral component of the autophagic pathway, in α-syn secretion and pathology, using stable cell lines that overexpress α-syn at lower levels. **Methods:** Exosomes were purified by sequential ultracentrifugation of conditioned medium of neuronal NSC-34 cells stably transfected with PD-linked α-syn (overexpressed WT, A30P, E46K, A53T). Stable cell lines were co-transfected with WT or dominant-negative (DN) endocytic Rab GTPases (Rab5, Rab7, Rab11 or Rab27) and analysed for exosomal α-syn secretion and ELS markers using immunoblotting and immunofluorescence microscopy. **Results:** Exosomes were the primary mechanism for endogenous α-syn secretion, with greater secretion of mutant α-syn than WT through both exosome and non-exosome dependent pathways. Increased α-syn expression induced autophagy markers p62, hsp70, LAMP2A and LC3, and accumulation of endocytic Rab proteins. Overexpression of WT or DN Rab11 enhanced or blocked α-syn secretion, respectively, implicating a recycling endosome pathway in α-syn release. **Conclusion:** These results point to exosomal dysfunction when α-syn expression is increased. This is demonstrated by elevated α-syn secretion, autophagic induction and endocytic Rab abnormalities. Understanding the secretory mechanisms of α-syn assists in the early diagnosis of pre-symptomatic PD patients, providing much needed insight into the early molecular mechanisms of PD.

POS-TUE-152

THE ROLE OF SEZ-6 IN NEUROPATHIC PAIN

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Pain of neuropathic origin afflicts ~8% of the general population and affected individuals suffer from conditions such as allodynia and hyperalgesia. The drugs gabapentin and pregabalin are used for treatment of neuropathic pain. Although they were predicted to act on receptors for GABA, the receptor for these drugs is now known to be an accessory subunit of voltage-sensitive calcium channels, α2-δ. Our new evidence suggests that α2-δ promotes the formation of excitatory connections between neurons through interacting with Seizure-related gene 6 (Sez-6) protein. Since blocking α2-δ is an effective treatment for neuropathic pain, we hypothesized that Sez-6 contributes to the synaptic gain-of-function in spinal cord dorsal horn neurons in neuropathic pain. We tested Sez-6-/- mice and controls for mechanical and heat-induced sensitivity, after which half of the mice underwent partial sciatic nerve chronic constriction (CCI) surgery, and the other half had the nerve exposed but not ligated (sham). Sez-6-/- mice showed significant increased sensitivity to heat-induced pain compared to control mice before surgery. Sensitivity to mechanical pain was increased two-fold in both control and Sez-6-/- mice 12 days after surgery. Golgi staining of the lumbar spinal cords was performed and neurons with characteristics of wide dynamic range neurons in sections from L4/L5 were analyzed. The results show a 50% increase in both the total number of dendritic spines per neuron in Sez6-/- mice with CCI, as well as an increase in mature spines, compared to control or sham-operated mice. These findings implicate Sez-6 in regulating the morphological plasticity occurring in response to increased excitatory drive in neuropathic pain.

POS-TUE-153

DYNAMICS OF CALCIUM MICRODOMAINS POST-INJURY IN OPTIC NERVE SUSCEPTIBLE TO SECONDARY DEGENERATION

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Purpose: Changes in calcium ion concentration are believed to play a vital role in the spread of secondary degeneration following injury to the CNS. We use novel nanoscale secondary ion mass spectrometry (NanoSIMS) to image changes in calcium ions in neurons and glia vulnerable to secondary degeneration *in vivo*. **Methods:** Partial optic nerve transection in PVG rats was used as a model of secondary degeneration. Rapidly excised segments (200µm) of nerve from normal animals or 1, 3, 7 days, 1 or 3 months post injury (n≥3) were cryopreserved and analysed using NanoSIMS. **Results:** Calcium ions were observed as microdomains which were divided into two categories: those associated with phosphorus (P), and those not. Microdomains are intracellular aggregates of calcium ions that may be located at the plasma membrane, in mitochondria and/ or endoplasmic reticulum. In normal uninjured glia, there was a greater proportion of non-P associated microdomains (P associated = 0.12 ± 0.05; non-P associated = 0.88 ± 0.05, p≤0.05). However, at 5 minutes, 1, 7 days, 1 or 3 months post-injury this difference of proportions was no longer significant. Interestingly, the predominance of P associated microdomains was not apparent in axons. A corresponding significant decrease in calcium content of glial non-P associated microdomains was also observed at 1 day post injury (p≤0.05). **Conclusions:** Our data indicate that in glial cells post-injury, there is an efflux of calcium out of non-P associated microdomains. The destination of the released calcium ions is currently under unclear.

POS-TUE-154

SUBCHRONIC METABOTROPIC GLUTAMATE 5 RECEPTOR MODULATION IN THE PERINATAL PCP RODENT MODEL OF SCHIZOPHRENIA

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Purpose: Schizophrenia is a complex neuropsychiatric disorder whereby symptoms present at adolescence. It is hypothesised the etiology of schizophrenia is due to NMDA receptor (NMDAR) hypofunction. Metabotropic glutamate 5 receptor (mGluR5) positive allosteric modulator (PAM) drugs are being investigated as a novel treatment of schizophrenia as an indirect manner to up-regulate NMDAR activity. We investigated the potential of subchronic adolescent CDPPB (an mGluR5 PAM) administration, to attenuate perinatal phencyclidine (PCP)-induced neurotransmission deficits. **Methods:** Male rat pups (n=6/group) were treated with PCP (10mg/kg) or saline on postnatal days (PN) 7, 9 and 11. Adolescent male rats (PN28) were administered with daily CDPPB (30mg/kg) injections for seven consecutive days (PN28-34) and euthanased on PN35. Subsequently [^{3}H]MK-801 and [^{3}H]MPEP radioligand binding were performed on brain sections corresponding to the pre-frontal cortex, striatum, thalamus, and hippocampus. **Results:** No significant differences were observed in NMDAR binding between any of the treatment groups in all brain regions examined. However mGluR5 binding density was significantly reduced by 31% in the ventral hippocampus of the PCP/CDPPB treated group compared to the saline control group (p=0.034). Similarly, mGluR5 binding density was significantly reduced by 49% in the striatum of the PCP/CDPPB treated group compared to the saline control group (p=0.01) and by 41% compared to the PCP/vehicle group (p=0.011). **Conclusion:** This study shows adolescent subchronic administration of CDPPB (30mg/kg) to have brain region specific effects on neurotransmission. Though we found reductions in mGluR5 binding density in CDPPB treated groups, this was not reflected in NMDAR binding density. Further investigation may prove this model a potential prevention tool to attenuate NMDAR hypofunction deficits.

POS-TUE-155

PHOSPHORYLATION OF α3 GLYCINE RECEPTORS INDUCES A CONFORMATIONAL CHANGE IN THE GLYCINE-BINDING SITE

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Purpose: Inflammatory pain sensitization is initiated by prostaglandin E2-induced stimulation of protein kinase A in spinal nociceptive neurons. This phosphorylates α3 glycine receptor (GlyR) chloride channels at Ser346, causing a reduction in glycinergic synaptic current magnitude and the subsequent disinhibition of nociceptive projection neurons. Drugs that specifically potentiate α3 GlyR currents should therefore have therapeutic efficacy as analgesics. Here we sought to compare glycine-induced conformational changes in α1 and α3 GlyRs to identify structural differences that might be exploited in the design of α3-specific therapeutics. **Methods:** GlyRs were recombinantly expressed in Xenopus oocytes and studied using two-electrode voltage-camp and fluorescence recording. All results were averaged from at least five independent experiments. **Results:** Using voltage-clamp fluorometry, we showed that glycine-mediated conformational changes in the extracellular M2-M3 channel gating domain were significantly different between the two GlyR isoforms. By using a chimeric approach, we found that structural variations in the large intracellular M3-M4 domain were responsible for this difference. This prompted us to test the hypothesis that phosphorylation of S346 (in the M3-M4 domain of the α3 GlyR) might also induce extracellular conformation changes. Surprisingly, phosphorylation modified glycine-induced structural changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. **Conclusions:** These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in any ligand-gated ion channel family member, and thus suggest new loci for investigating how phosphorylation modulates structure and function in this receptor family. Second, by demonstrating that inflammatory pain sensitization confers a unique conformational change in the α3 GlyR glycine-binding site, they raise the possibility of developing novel analgesic drugs that selectively target disease-affected GlyRs.

POS-TUE-156

INFLUENCING EXCITOTOXICITY: A ROLE FOR TYPE 1 INTERFERONS

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Introduction: Neuroinflammation and excitotoxicity are processes associated with acute and chronic neuropathologies. Evidence suggests that neuroinflammation can influence levels of excitotoxicity. Type-1 IFNs are major regulators of the inflammatory cascade and activation of the type-1 IFN pathway leads to the up-regulation of NFκB, a transcriptional mediator of various pro-inflammatory cytokines and chemokines. Interestingly, key subunits of the NMDA receptor contain an NFκB promoter binding site suggesting that a pro-inflammatory response leading to increased NFκB activation could influence NMDA receptor expression. We investigated the role of the type-I IFNs in mediating NMDA receptor-mediated toxicity.**Method and Results:** NMDA receptor expression was investigated by immunohistochemistry and western blot analysis in wildtype and Interferon-α receptor-1 (IFNAR1)$^{-/-}$ mice, which display reduced type-I IFN singalling. Immunohistochemical analysis revealed decreased levels of NMDAR2A staining in IFNAR1$^{-/-}$ brain sections compared to WT. Western blot analysis confirmed a significant decrease in the expression of NMDAR1 (1.76 ± 0.59 vs 0.39 ± 0.06, p=0.0451, n=6), NMDAR2A (1.16 ± 0.28 vs 0.33 ± 0.08, p=0.0459, n=3) and NMDAR2B (1.03 ± 0.20 vs 0.13 ± 0.07, p=0.003, n=5) in the IFNAR1$^{-/-}$ brain compared to their wildtype counterpart. In addition, M17 neuroblastoma cells stably expressing an IFNAR1shRNA construct (IFNAR1KD) were used to investigate differences in cell viability following excitotoxic insult to L-glutamate. A significant, protective effect was observed in the IFNAR1KD cells compared to negative control (shRNA) cells following 15mM (65.06 ± 3.99% versus 39.92 ± 6.85%) and 20mM (37.11 ± 9.98% versus 17.68 ± 7.53%) glutamate treatment (24 hours). **Conclusion:** We propose that reduced type 1 IFN signaling leads to decreased NMDA receptor expression, and thus a decreased capacity for excitotoxic cell death following neural insult.

POS-TUE-157

TAM EXPRESSION IS DIFFERENTIALLY REGULATED IN IMMUNE CELLS DURING INFLAMMATORY DEMYELINATION

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Background: The TAM family of receptor tyrosine kinases (Tyro3, Axl and Mertk), and their ligands (Gas6 and Protein S (ProS)), have been shown to regulate both neural and immune responses during central demyelination. Here, we investigate the expression of TAM receptors and ligands in the CNS and peripheral immune cells using a model of inflammatory demyelination. **Methods:** Experimental autoimmune encephalomyelitis (EAE) was induced in C57Bl/6 mice (n=3-6). Spinal cords and spleens were collected from mice at days 0, 8, 15 and 21 post-induction. **Results:** Using qPCR, we observed increasing expression of Axl, Mertk and ProS with EAE progression in the CNS ($p<0.001$), whilst Tyro3 and Gas6 expression decreased ($p<0.001$). In CD11b+ monocytes we observed downregulation of Axl and Gas6 expression ($p<0.001$) and a transient increase in Tyro3 expression at EAE day 8 ($p<0.05$). In contrast in CD11c+ dendritic cells, we observed upregulation of Axl and Mertk expression until EAE day 15 ($p<0.05$), returning to baseline levels at day 21. Using flow cytometry, we found ~80% of T-cells and B-cells express Tyro3 at all timepoints examined. Mertk expression was downregulated on T-cells with EAE progression, with a transient increase in expression at EAE day 8 on B-cells. **Conclusions:** These data show differential regulation of TAM expression in innate immune cell subtypes and modulation of TAM receptors on adaptive immune cells during EAE, providing evidence that TAM signalling may modulate central demyelination by regulating immune responses, both innate and adaptive. Future work will examine conditional deletion of individual TAM receptors from innate immune cell subtypes.

POS-TUE-158

CHANGES IN BRAIN EDEMA AND INTRACRANIAL PRESSURE FOLLOWING TRAUMATIC BRAIN INJURY ACROSS THE ESTROUS CYCLE: INVOLVEMENT OF FEMALE SEX STEROID HORMONES

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ABSTRACT Purpose: It has shown that sex steroid hormones have profound neuroprotective effects in experimental traumatic brain injury (TBI). Because the endogenous hormone levels are proven to differ with estrous cycle stage, we evaluated whether estrous cycle stage affects various outcomes following diffuse TBI. Methods: TBI was induced by the Marmarou's method in normal cycling and in ovariectomized rats (n=7) with physiologically relevant restoration of hormonal levels by hormone capsule implantation. Intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were measured before and different times after TBI and brain edema was assessed at 24 h after trauma. Results:Results indicated that after TBI, water content (WC) in traumatic proestrous (TP) rats was less than the one in traumatic non-proestrous (TNP) and ovariectomized (TOVX) and also in high estradiol (HE) and progesterone (HP) was statistically less than TBI untreated groups. There was no significant difference in WC between high doses hormone treated and TP and also between TNP, TOVX, low estradiol (LE) and progesterone (LP) groups. At 4 h and 24 h after trauma, there was a significant difference in ICP between TP, HE and HP compared to TNP and other TBI nontreated groups. Also in these times, the CPP was increased in TP and hormone treated groups compared with TOVX, but the difference between TNP and TOVX was not significant. Conclusion: The results indicate that the estrous cycle has a prominance role in TBI outcome's and the difference in female sex steroid levels might be the reason of the different neuroprotective effects in proestrous and non-proestrous groups.

POS-TUE-159

TYPE-1 INTERFERON SIGNALLING CONTRIBUTES TO THE NEUROINFLAMMATORY RESPONSE IN PARKINSON'S DISEASE

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Purpose: Neuroinflammation contributes to the neuronal cell death in Parkinson's disease (PD). Key players in the neuroinflammatory cascade are the type-I Interferons (IFNs), however their role in PD is unknown. We propose that the type-I IFNs contribute to the progression and exacerbation of neuronal cell death in PD. **Methods and Results:** This study investigated type-I IFNs in post mortem human brain tissue, and *in-vitro* using human BE(2)M17 neuroblastoma (M17) cells treated with the PD associated neurotoxin, rotenone. QPCR analysis of pre-frontal cortex confirmed a 3- and 4-fold up-regulation of IFNα and IFNβ in PD patients, compared to age matched controls (n=10, *$p<0.05$). *In-vitro* studies confirmed rotenone (10nM-1μM) induced cytotoxicity in M17 cells, associated with activation of type-I IFN signalling with western blot analysis confirming increased STAT-3 phosphorylation. In parallel, an up-regulation of IFNα (30-fold, n=5) and IFNβ (5-fold, n=5) was identified by QPCR. Additionally, cells displayed an upregulation in IFN-regulated genes IRF3 and RIG-I, and pro-inflammatory cytokines IL-1β, TNF-α and IL-6. Significantly, stably expressing M17 Interferon Receptor-1 (IFNAR1) knockdown cells, showed significantly reduced levels of IFNα, IFNβ, IL-1β and TNF-α compared to negative control shRNA (NC-shRNA) cells following rotenone treatment (*$p<0.05$, n=5). IFNAR1 knockdown cells exhibited decreased cell death induced by 500nM rotenone compared to NC-shRNA cells (83.7±4.2% vs. 66.1±2.9% n=6, $P<0.05$). Western blot analysis revealed that this protection was also associated with a significant decrease in cleaved caspase-3 in IFNAR1 knockdown cells. **Conclusion:** These results implicate type-I IFNs in both post mortem human tissue and cellular models of PD. Our data suggests that targeting type-I IFN signalling may reduce neuroinflammation and thereby limit the neuronal cell death in PD.

POS-TUE-160

SUPEROXIDE GENERATION AND CEREBRAL VESSEL DENSITY IN NOX2 KNOCKOUT MICE FOLLOWING ISCHAEMIC STROKE

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Purpose: NADPH oxidase-derived reactive oxygen species (ROS) are thought to contribute to the progression of brain injury following stroke. We examined the role of the Nox2 oxidase in ROS generation and cerebral blood vessel density following ischaemia and reperfusion in the brain using knockout mice. **Methods:** The middle cerebral artery was occluded via intraluminal filament for 1 h followed by 6, 24 or 72 h recovery in Nox2-/- and Nox2+/+ mice. ROS were detected *in-situ* using dihydroethidium (DHE) fluorescence and localized using double-labelling with antibodies against neurons and macrophages. Blood vessel density was quantified using immunohistochemistry. **Results:** Infarct volume was not different between Nox2+/+ and Nox2-/- mice at 6 h post-stroke ($P=0.471$), but was significantly reduced in Nox2-/-, compared with Nox2+/+ mice at 24 h post-stroke ($P<0.01$). A delayed increase in Nox2-/- infarct size resulted in no difference between genotypes at 72 h ($P=0.248$). Following 6 h of reperfusion, DHE-detected superoxide was increased in the stroke-affected cortex and striatum of both Nox2-/- ($P<0.001$) and Nox2+/+ ($P<0.001$) mice compared to control regions. This increase was significantly greater in Nox2+/+ than Nox2-/- mice ($P<0.001$) and localised to both neurons and inflammatory cells. At 72 h post-stroke, blood vessel density was decreased in Nox2+/+ mice ($P<0.001$), but had returned to control levels in Nox2-/- mice, resulting in a significant difference between genotypes ($P<0.05$). **Conclusion:** The current results suggest that genetic inhibition of the Nox2 oxidase attenuates the increase in ROS generation detected at 6 h post-stroke and delays brain damage following ischaemia and reperfusion. Nox2 inhibition appears to be of benefit in allowing blood vessel density to return to control levels at 72 h post-stroke.

POS-TUE-161

AUTOPHAGY IN ECSTASY-INDUCED INJURY: A NEUROPROTECTIVE TARGET TO MANAGE TOXICITY OF SEROTONERGIC NEURONES?

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The addictive and toxic effects of ecstasy (3,4-methylenedioxy-methamphtamine, MDMA) via actions on biogenic amine neurones are well documented. While oxidative stress, DNA damage and ubiquitinated inclusions may all contribute to MDMA-mediated neurotoxicity, difficulties associated with primary culture of serotonin (5-HT) neurones have handicapped efforts to delineate its injury mechanisms. **PURPOSE**: To explore the profile of MDMA toxicity in a primary culture containing 5-HT neurones. **METHOD:** Tissue containing rostral raphe nuclei (E14-16 mice) was digested and isolated cells plated in microwell plates or on glass coverslips (0.1-0.2 x 10^6 cells/well). **RESULTS:** Cytochemistry (12 *div*; n>3) indicated a MAP-2, 5-HT-immunopositive population of cells exhibiting extensive neuritic trees with large primary axons. MTT assays and measurements of [^{3}H]5-HT uptake (n=3) indicated recruitment of programmed cell death in a concentration-dependent manner by oxidative stressor (hydrogen peroxide; IC_{50} 100 µM), autophagic stressor (rapamycin; IC_{50} 15 µM) and MDMA (100 - 1000µM). 5-HT-positive neurones underwent dieback of their neuritic trees involving nuclear (Hoechst) and DNA (TUNEL) fragmentation in an insult-dependent manner. Western immunoblotting (n=2) for microtubule associated protein light chain 3 (LC-3) revealed conversion of LC3-I to LC3-II consistent with autophagosome formation with both rapamycin and MDMA. Confocal analyses (n=2) after cytochemistry for 5-HT, Hoechst and LC3 indicated MDMA increased autophagic activity as shown by abundant LC3-positive puncta within 5-HT neurones, apparently after initiation of nuclear fragmentation. **CONCLUSION:** MDMA possesses the capacity to induce autophagy and its multifaceted nature makes it amenable to pharmacological manipulation to decrease MDMA neurotoxicity of 5-HT neurones.

POS-TUE-162

IRON AND NEUROPATHOGENESIS - INSIGHTS FROM MOUSE MODELS OF IRON OVERLOAD

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Purpose: To examine effects of iron overload on the brain using mouse models of the common iron disorder haemochromatosis (prevalence over 1 in 1,000). **Methods:** We used wildtype mice, two single mutant models (Hfe$^{-/-}$; Tfr2^{mut}) and double mutant Hfe$^{-/-}$xTfr2^{mut} mice, all on AKR background (ages 12-52 weeks; normal chow or short-term high iron diet). We assessed brain iron by inductively coupled-atomic emission spectroscopy (ICP-AES), Perl's stain and non-haem iron assay, transcript changes by microarray and real-time RT-PCR and protein changes by immunoblotting and immunohistochemistry. **Results:** Brain iron measures did not differ from controls in single mutant and iron supplementation models but were over 70% higher in Hfe$^{-/-}$x Tfr2^{mut} double mutant mice ($p<0.025$, n≥5/group). Various important transcripts showed changes in two or more models, including Fos and CamK2α. All models showed transcript changes relating to lipofuscin diseases but only Hfe$^{-/-}$xTfr2^{mut} mice showed strong evidence for oxidative damage, such as lipid peroxidation. Although all genetic models displayed changes for Alzheimer's disease-related transcripts (e.g. Notch/presenilin), none showed evidence for increased amyloid precursor protein (APP) transcripts, protein or changes in neuronal expression in response to iron overload, raising questions about claims that APP is the neuronal ferroxidase. **Conclusion:** The findings substantiate haemochromatosis patient complaints of brain-related problems. Important brain molecular components are considerably affected even by mild iron dyshomeostasis, with probable consequences for many brain conditions.

POS-TUE-163

TYPE-1 INTERFERONS PROPAGATE NEURO-INFLAMMATORY CASCADES IN MODELS OF ALZHEIMER'S DISEASE

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Purpose: Neuro-inflammation has recently been implicated in Alzheimer's disease (AD) pathology. Type-1 interferons (IFNs) are involved in the regulation of neuro-inflammation, however their role in disease progression is unknown. Type-1 IFNs bind their receptor, IFNAR1 activating the JAK/STAT cascade, recently implicated as a mediator of soluble Aβ1-42 toxicity (Wan et al., 2010). This study investigated the contribution of type-1 IFN signalling to the neuro-degeneration in AD using *in vivo* and *in vitro* models. **Methods & Results:** APP/PS1 brains (9 months) showed a significant 2-fold increase in IFNα protein levels by ELISA compared to aged matched controls (n=4, $P<0.05$). Immunohistochemistry identified elevated STAT-3 phosphorylation (a downstream mediator of type-1 IFN signalling) in neurons (FOX-3a positive) of the frontal cortex of APP/PS1 brains (n=5). This staining surrounded amyloid plaques, accompanied by elevated astrogliosis (GFAP). We previously demonstrated that IFNAR1$^{-/-}$ neurons are protected following Aβ1-42 insult through decreased IFN production and pro-apoptotic caspase-3 activity. To further characterise the specific cellular responses involved in AD, primary cultured glia were treated with Aβ1-42. Wild-type glia demonstrated a 2-fold increase in both IFNα and IFNβ mRNA by Q-PCR after Aβ1-42 insult whilst levels in IFNAR1$^{-/-}$cultures remained unchanged (10µM, 48hrs, n=3, $P<0.05$). Previously, IFNAR1$^{-/-}$ neurons showed reduced IFNβ, not IFNα, mRNA levels compared to wild-type in response to Aβ1-42 insult. **Conclusion:** This study supports a role for type-1 IFN signalling in the pro-inflammatory response that is generated by Aβ. The data suggests that glia alongside neurons are involved in producing IFN in response to Aβ. Therefore blocking IFNAR1 signalling may be beneficial in reducing neuro-inflammation and neuro-degeneration in AD.

POS-TUE-164

DENDRITIC CELL CCAAT/ ENHANCER BINDING PROTEIN DELTA MODULATES TH17/TREG RESPONSES IN AN IL-10 DEPENDENT MANNER

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Purpose: CCAAT/enhancer binding protein delta (C/EBPδ), a βZIP-transcription factor, is an emerging regulator of innate immune responses in central nervous system (CNS) autoimmune disease. However, it hasn't been examined in this context. This study aimed to define the actions of dendritic cell (DC) C/EBPδ in experimental autoimmune encephalomylitis (EAE). **Methods:** EAE and bone marrow (BM) chimeric animals were used to determine the contribution of systemic circulation derived DC-C/EBPδ to CNS inflammation. *In vitro* and *in vivo* models of DC directed T-cells activation were examined by fluorescent activated cell sorting, multiplex immunoassay and qPCR to determine secreted cytokines mediating DC-C/EBPδ effects. Finally, inhibitory antibody treatment both in vitro and in vivo confirmed the role of indentified cytokines. **Results:** Induction of EAE lead to the upregulation of C/EBPδ mRNA expression in CNS astrocytes and DCs. Further, C/EBPδ knockout mice had significantly reduced EAE severity. Reduced EAE severity was attributable to isolated knockout of C/EBPδ in circulating immune cells, suggesting reduced DC C/EBPδ expression was responsible for alleviation of disease. Reduced DC expression of C/EBPδ led to increased anti-inflammatory T-regulatory polarisation, at the expense of pro-inflammatory Th17 development, both *In vitro* and *in vivo*, and Th1 development was unaffected. Additionally, lack of DC-C/EBPδ expression was associated with increased IL-10 transcription and secretion. Finally, the inhibition of IL-10 actions by a specific anti-IL-10 receptor antibody reversed the effect of absent DC-C/EBPδ both *in vitro* and *in vivo*. **Conclusion:** DC expression of C/EBPδ regulates IL-10 expression and is an important regulator of Th17:Treg balance.

POS-TUE-165

THE BETA-AMYLOID PROTEIN-INDUCED PHOSPHORYLATION OF CRMP-2 AND ITS CONTRIBUTION TO NEURONAL DYSFUNCTION

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Purpose: Alzheimer's disease (AD) is an age-related neurodegenerative disorder and the most common form of dementia in the elderly. The hallmarks of AD pathology are the amyloid beta (Abeta) polypeptide extracellular deposition and formation of intracellular neurofibrillary tangles (NFTs), along with dystrophic neurites. Evidence suggests that oligomeric Abeta can induce neuritic dystrophy. The purpose of this study is to investigate how Abeta regulates the microtubule associated protein, collapsin response mediator protein (CRMP-2), by phosphorylation, thereby limiting neurite growth. **Methods:** Post-mortem temporal and frontal lobe cortical AD brain lysates (n=4 patients), along with fronto-temporal dementia (FTD) (n=5 patients) and non-neurological disease control brain lysates (n=2 patients) were analysed by western blotting to identify CRMP-2 phosphorylation. Coronal brain sections from Tg2576 mice (n=12) were immunostained for phospho-Thr555 CRMP-2 and data compared with wild type brains (n=4). SH-SY5Y cells were also transfected with CRMP-2 phosphorylation mutant constructs (n=5 constructs), treated with Abeta (0.5, 1.0 and 10uM for 24h), to define which Abeta-mediated kinase activity may initiate phospho-CRMP-2 dependent neurite retraction. **Results:** Human brain lysates show increased PThr555CRMP-2 levels in AD compared with FTD and control samples. Cortical and hippocampal neurons from aged Tg2576 mice (12-18 months) demonstrated substantial staining especially in hyperphosphorylated tau-positive neurons. Moreover, SH-SY5Y cells transfected with the T555A phospho-CRMP-2 mutant construct generated larger processes when compared to cells transfected with the other constructs (sites phosphorylated by other kinases). **Conclusion:** These data suggest that the phosphorylation of the Thr555 site of CRMP-2 may be central to Abeta-dependent neurite abnormalities associated with AD pathology.

POS-TUE-166

CATECHOL-O -METHYL TRANSFERASE IS SELECTIVELY UPREGULATED IN THE PERIAQUEDUCTAL GREY OF RATS FOLLOWING SCIATIC NERVE INJURY

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Purpose: The periaqueductal grey (PAG) receives a large ascending catecholaminergic input from both adrenergic and noradrenergic brainstem nuclei. These nuclei undergo adaptation and increase their activity following sciatic nerve injury. A likely consequence of this is increased catecholaminergic (CA) drive on PAG neurons. Sciatic nerve injury triggers pain in all rats, however, in a subset there are altered behavioural and endocrine responses akin to the disabilities reported in human chronic pain populations. We asked the question whether the CA drive on the PAG was the same in rats, with and without disabilities by evaluating the expression of the CA inactivation enzyme catechol-o-methyl transferase (COMT). **Methods**: COMT mRNA and COMT protein expression levels were determined in the PAG of rats with (N=12) and without (N=15) disability following nerve injury, defined by reductions in dominance in a resident-intruder, social interaction test. RT-qPCR was used to quantify mRNA levels. Western blots were used to quantify COMT protein levels and standard immunohistochemical techniques were used to anatomically localize, regional COMT expression in serial sections of midbrain. **Results**: COMT mRNA expression was significantly up-regulated in rats with disability ($p<0.05$), similarly COMT (24kD & 28kD) protein levels were significantly ($p<0.05$) greater in rats with disability. COMT immunoreactive profiles were located predominantly in the ventrolateral PAG, and were significantly denser in the caudal ventrolateral PAG of rats with disability. **Conclusion**: The significant up-regulation of COMT in the ventrolateral PAG of rats with disability and pain following nerve injury, suggests that reducing CA drive on neurons in this region, in the injured state may underlie the expression of disability in a this subset of rats.

POS-TUE-167

POTENT ANTI-INFLAMMATORY EFFECTS OF ANDROGRAPHOLIDE AND ITS MAJOR METABOLITE, ANDROGRAPHOLIDE SULFONATE

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Purpose: Chronic inflammation is a contributing factor for many ageing-related diseases including Alzheimer's disease (AD). In order to provide effective, yet safe anti-inflammatory treatments, there is a renewed interest in the search of plant based novel secondary metabolites. Andrographolide, an ent-labdane diterpene from an ayurvedic herb Andrographis paniculata has been traditionally used for the treatment of chronic inflammatory diseases. However, andrographolide exhibits poor bioavailability (< 3%), and is known to rapidly metabolize to a sulfonate with unknown potency, which was investigated in this study. Methods: Anti-inflammatory activity was determined by nitric oxide production in LPS + IFN activated RAW264.7 macrophages (n=3, in triplicate). Cell viability was measured using the MTT reduction assay (n=3, in triplicate), Results: Andrographolide and its major metabolite, andrographolide sulfonate both demonstrated strong anti-inflammatory activity with IC (50) values of 12.4 ± 0.6 μM and 14.2 ± 0.3 μM, respectively. Both compounds were much more potent than the NSAIDs aspirin and ibuprofen or paracetamol (IC(50) values > 1 mM). The LC (50) concentrations for andrographolide and andrographolide sulfonate were determined to be 272 ± 20 μM and 489 + 11 μM, respectively. Conclusion: The nearly equipotent anti-inflammatory activity of andrographolide sulfonate (which exhibits > 20 times higher plasma levels than andrographolide), together with its extended half-life, might account for its purported clinical efficacy.

POS-TUE-168

EFFECTS OF *IN UTERO* BISPHENOL A EXPOSURE ON BRAIN AND BEHAVIOUR

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Background: Bisphenol A (BPA) is an oestrogenic industrial chemical used in the manufacture of polycarbonate plastic. Evidence from fertility, behaviour and development studies in rodents and humans illustrates its harmful effects. **Aim:** To test the effect that *in utero* BPA exposure on the brain and behaviour. Methods: We subcutaneously injected pregnant FVBN wild-type female dams to varying doses of BPA (vehicle, 25, 50 and 100 ug/kg/day BPA) everyday for the entire course of their gestation (19 days) and analysed the behaviour and brain phenotype of their offspring. The social interaction of the weaned pups was analysed using the 3 chamber social behaviour test, a robust test for social approach and preference for social novelty (or social recognition). **Results:** We found that offspring of 100 BPA-treated dam spent less time in the novel arm vs home arm compared to age matched offspring from vehicle-treated dams in the Y maze test (2 way ANOVA; $p<0.001$). Thus, this indicates that offspring of BPA exposed dams show short-term spatial memory deficits. We found that offspring from vehicle-treated dams showed social novelty preference (Mann Whitney test; $p=0.01$) whilst this behaviour is abolished in a BPA dosage-dependent manner, with the offspring of 100 BPA-treated dams spend equivalent time (s) interacting with the 'novel' stranger and the 'familiar' mouse. Thus, indicating that progeny of BPA treated dams may have social recognition deficit. Stereological analysis is being undertaken to understand the effect of BPA on the brain morphology. **Conclusion:** *In utero* exposure of BPA resulted in social behavioural impairment and spatial memory deficits.

POS-TUE-169

MICRORNAS ASSOCIATED WITH A MODEL OF RETINAL DEGENERATION

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PURPOSE: MicroRNAs (miRNAs) are implicated in a number of neurodegenerative disorders. We aim to identify the microRNAs involved in retinal degenerations (RD) using a model of Dry Age Related Macular Degeneration (AMD). **METHODS:** Sprague-Dawley (SD) rats were exposed to 1000 lux light for 24 hours. Retinal RNA was extracted (n=5) in each of the two experimental groups (Control, Light Damage [LD]). cDNA was hybridized to TaqMan Rodent miRNA array cards (Life Technologies) to quantify 750 unique miRNAs (published in an open access miRNA database - miRBase). The results obtained were collectively analysed along with some previously published microarray data (Natoli *et al*, 2010) identifying messenger RNAs (mRNAs) modulated by LD. Bioinformatics was performed using the Partek Genomics Suite 6.6. **RESULTS:** High-throughput Real-time quantitative PCR accompanied by stringent normalisation and filtering strategies facilitated the identification of 37 miRNAs differentially expressed by LD, of which 26 were up-regulated and 11 down-regulated. Collective analysis (miRNA-mRNA interactions) identified 20 novel retinal miRNAs. Subsequent analysis using Gene Ontology identified 3 functional clusters modulated by LD - metabolic process (including catalysis of the oxidation-reduction process), cellular process (including ligand-receptor binding) and response to stimulus (including immune response). **CONCLUSIONS:** Oxidative damage and inflammation are associated with RD including AMD. We have identified a number of potential miRNAs that may be involved in the pathogenesis of RD.

POS-TUE-170

HYPOXIC PRECONDITIONING AND CELL PROLIFERATION IN A NEONATAL RAT MODEL OF HYPOXIC-ISCHAEMIC BRAIN INJURY

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Purpose: Neonatal hypoxic-ischaemic brain injury remains a significant cause of death and long-term neurological disability in children. Neural stem/progenitor cells (NPCs), which exist in developing and adult brain, are self-renewing and can differentiate into neurons, astrocytes or oligodendrocytes, providing great potential for regenerating lost cells after brain injury. One possible neuro-restorative strategy to activate endogenous brain repair is hypoxic preconditioning (HP). We investigated whether HP can increase the proliferation of NPCs in newborn rat brain. **Methods:** Sprague-Dawley rat pups (Postnatal day (P) 6) were subjected to HP (8% O2; 3 hours) and normoxic rats (n=5) were maintained in room air. On P7, rats were subjected to hypoxic-ischaemic (HI) injury. Bromodeoxyuridine (BrdU 50mg/kg, IP) injections were performed twice daily, for 3 days after HI, to label proliferating cells. On P10, brains were removed for histological analysis. Immunohistochemical staining for BrdU and double-immunostaining with fluorescence microscopy were performed to determine cell phenotypes. **Results:** HP animals (n=9) showed a significant one-fold increase in the number of BrdU-positive cells in the cortex compared with HI (n=18) and HP+HI (n=19). Additionally, no difference in cell numbers was observed in the dentate gyrus and subventricular zone (P>0.05, 1-way ANOVA). Co-labeled BrdU-positive cells with neuronal (NeuN) and astrocyte (GFAP) markers were observed across all treatment groups. However, there were no differences between groups regarding NPCs phenotypes (P>0.05, 1-way ANOVA). **Conclusion:** These results suggest that cell proliferation is at its peak at P10 and none of the treatments further increased NPCs proliferation and maturation. These findings should be considered when developing therapeutic interventions to enhance endogenous neurogenesis following newborn brain injury.

POS-TUE-171

VASCULAR DEGENERATION IN PARKINSON'S DISEASE

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Purpose: Vascular degeneration has been identified as a significant contributor to the neurodegenerative process in Alzheimer's disease but our understanding of the vascular components in Parkinson's disease is limited. **Aim:** To investigate the vascular contribution to Parkinson's disease progression. **Methods:** Vascular morphology was examined in human brain tissue from a number of regions in Parkinson's disease (16 cases) and controls (10 cases). Immunohistochemical staining, using von Willebrand factor as a marker of endothelial cells, and ImageJ analysis was used. A two-way ANOVA was used to compare Parkinson's and control cases and the Bonferroni post-test used for specific differences between individual brain regions. **Results:** The degenerative morphology seen in Parkinson's disease cases included the formation of endothelial cell 'clusters' that may well contribute to the fragmentation of the capillaries. When compared to control cases, the capillaries of Parkinson's disease brains were less in number ($p<0.001$), shorter in length ($p<0.001$) and larger in diameter ($p<0.01$) with obvious damaged to the capillary network evidenced by less branching ($p<0.001$). Vessel degeneration associated with Parkinson's disease was found in multiple brain regions, but particularly in the substantia nigra, middle frontal cortex and brainstem nuclei (locus coeruleus and Raphe), but was less evident in the caudate nucleus. The degree of degeneration seen in the caudate nucleus was also apparent in the age matched control cases. **Conclusions:** Our data suggests that vascular degeneration may be a contributing factor to the progress of Parkinson's disease. Thus preventing vascular degeneration and improve vascular remodelling may afford a novel approach for the treatment of Parkinson's disease.

POS-TUE-172

CLINICALLY RELEVANT HUNTINGTON'S DISEASE MUTATIONS DO NOT PERTURB NEURAL DEVELOPMENT OF HUMAN EMBRYONIC STEM CELL LINES

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Purpose: Huntington's Disease (HD) is an incurable neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the Huntingtin gene. Recently, induced pluripotent stem cell lines carrying atypical and aggressive (CAG60+) HD variants have been generated, and perplexingly exhibit disparate molecular pathologies. Here we investigate two human embryonic stem cell (hESC) lines carrying CAG35 and CAG51 mutations to assess whether clinically relevant expansions exhibit HD pathologies. Methods: HD hESC pluripotency, proliferation and viability were assessed in comparison to two wildtype control lines (H9 and HES3). Forebrain neuronal differentiation was examined concomitant with the expression levels of genes known to be dysregulated in HD. Further, mitochondrial and neuronal functional activities were assessed with JC-1 staining and glutamate stimulation respectively. Results: Pluripotent HD lines demonstrate growth, viability, pluripotent gene expression, mitochondrial activity and forebrain specification indistinguishable from control lines. While expression profiles of key genes remained unperturbed in the presence of mutant protein and throughout differentiation, abnormal glutamate responses ($n=3$, $p<0.01$) were observed in HD neurons. Conclusion: These findings suggest typical late-onset HD mutations do not alter pluripotent parameters or differentiation mechanics but that neuronal progeny may possess the capacity to recapitulate the various neuropathologies seen in human patients. Such HD models will help further our understanding of the cascade of pathological events leading to disease onset and progression, while simultaneously facilitating the identification of candidate HD therapeutics.

POS-TUE-173

IMMUNOGLOBULINS IN FRONTOTEMPORAL LOBAR DEGENERATION

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Purpose Immune reactions are a widely accepted pathological characteristic of many neurodegenerative diseases. Alzheimer's 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 (IG) proteins in FTLD tissue. Differences were observed between the tau-positive and tau-negative subtypes of FTLD which suggest different underlying immune processes. **Methods** Following institutional approvals, tissue was obtained from the Sydney Brain Bank: normal controls (n=11), FTLD (n=13) and AD (n=9). The immunoglobulins IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE were quantified in extracts from fresh frozen tissue from the superior temporal gyrus via Multiplex ELISA. IgG was also immunohistochemically evaluated in FTLD cases and normal controls. **Results** Tau-negative FTLD and AD cases showed significantly higher levels of IgG compared with Tau-positive FTLD cases ($p<0.05$). Levels of IgG in AD cases were significantly higher than controls ($p<0.05$). Similar differences were also observed in the density of IgG positive neurons using immunohistochemistry ($p<0.05$). **Conclusion** These findings indicate that the immune reactions not only differ between tau-positive and tau-negative FTLD cases, but also between tau-positive neurodegenerative dementias. This further suggests that mechanisms independent of aggregated, hyperphosphorylated tau influence inflammatory processes in these dementias. Previous work in our laboratory suggests that cytokine levels also differ between neurodegenerative dementias. Together, these results may indicate that different immunological pathways may be activated in FTLD and AD.

POS-TUE-174

NK1 RECEPTOR ANTAGONISTS AMELIORATE NEUROINFLAMMATION FOLLOWING TRAUMATIC BRAIN INJURY

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Purpose: Neuroinflammation can arise through compromised blood-brain barrier function. We have demonstrated that NK1 antagonists are able to restore BBB function following traumatic injury (TBI). The present study used a rodent model of TBI to assess whether post-injury administration of an NK1 antagonist could ameliorate the associated neuroinflammation. The effect of post-injury administration of an NK1 antagonist on serum IL-6 levels, as well as the cortical levels of IL-1β, IL-6 and TNFα mRNA were assessed. **Methods:** Adult male Sprague-Dawley rats (n=20) were anesthetized with isoflurane, and subject to either a sham injury (n=10) or a severe TBI (n=10). Severe TBI was induced using the impact-acceleration model of injury. Post-injury, animals received either an NK1 antagonist (N-acetyl tryptophan; 2.4mg/kg) or drug vehicle (n=5/group). Animals were killed 6 hours after injury, serum samples collected and brains excised. Serum levels of IL-6 were determined by ELISA, whilst TaqMan gene expression assays for IL-6, IL-1β and TNF-α were used. Statistical significance was determined using one-way analysis of variance (ANOVA). **Results:** In sham-injured animals, serum IL-6 levels were 103.7 ± 14.7pg/ml. Animals subject to TBI and drug vehicle treatment showed a significant rise in IL-6 levels (289.3 ± 75.9pg/ml; $p < 0.01$). However, animals treated with an NK1 antagonist 30mins after injury, showed no significant rise in serum IL-6 levels (127.6 ± 11.7pg/ml). RT-PCR revealed significant increases in mRNA levels of IL-6, IL-1β and TNF-α (134-, 32- and 45-fold respectively; $p < 0.01$). Animals treated with the NK1 antagonist showed no significant increase in mRNA levels of these mediators. **Conclusions:** This study indicates that post-injury administration of an NK1 antagonist may ameliorate neuroinflammation following TBI.

POS-TUE-175

TESTOSTERONE MODULATION OF DOPAMINE RECEPTORS IN THE SUBSTANTIA NIGRA AND DORSAL STRIATUM OF ADOLESCENT MALE RATS

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The disproportionate effect and earlier onset age of schizophrenia in males suggest a relationship between adolescent testosterone and disease pathogenesis. Increased dopamine activity in the nigrostriatal pathway underlies psychotic symptoms of schizophrenia. Two families of dopamine receptors exist: excitatory DRD1-like (DRD1, DRD5) and inhibitory DRD2-like (DRD2, DRD3, DRD4). Current antipsychotic treatments block DRD2 to alleviate psychosis but are limited in effectiveness. Testosterone acts through androgen receptor directly or after conversion to dihydrotestosterone (DHT) and through estrogen receptor after aromatisation to 17β-estradiol (E2) to control transcriptional activity of target genes. **Purpose:** To determine if sex steroids modulate dopamine receptor mRNA expression in the nigrostriatal pathway of male rats over adolescence and whether this is via androgenic or estrogenic mechanisms. **Methods:** Pre-adolescent (45 day old; 11-16/group) male rats underwent sham surgery or gonadectomy and were given testosterone, DHT or E2 replacement during adolescence for 14 days. Dopamine receptor mRNA levels were analysed in the substantia nigra (SN) and dorsal striatum. **Results:** In the SN, androgen replacement increased DRD2 and decreased DRD3 mRNA. DRD1 expression was increased by only DHT whilst DRD5 was increased by all three sex steroids. In the striatum, DRD2 mRNA was increased by all three sex steroids. Gonadectomy increased DRD5 expression which was attenuated by only E2 replacement. DRD1 and DRD3 expression were unchanged by treatment. **Conclusion:** Testosterone modulates dopamine receptor expression in the nigrostriatal pathway, mainly via androgenic mechanisms. Testosterone modulation of dopamine sensitivity of the nigrostriatal pathway may involve receptor-driven regulation of feedback inhibition in the region of the dopamine cell bodies and dopamine action in the striatum.

POS-TUE-176

THE ROLE OF PROTEIN DISULPHIDE ISOMERASE AND FAMILY MEMBERS IN AMYOTROPHIC LATERAL SCLEROSIS

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Purpose Amyotrophic lateral sclerosis (ALS) affects motor neurons of the brain stem and spinal cord. The cellular and molecular mechanisms underlying neurodegeneration in ALS are unclear but involve oxidative stress, protein aggregation, endoplasmic reticulum (ER) stress and apoptosis. Mutations in superoxide dismutase 1 (SOD1) cause 20% of familial ALS cases. We previously demonstrated that over-expression of protein disulphide isomerase (PDI) is protective against inclusion formation, ER stress and apoptosis in cells expressing mutant SOD1. PDI is a chaperone found in the endoplasmic reticulum (ER), prototype of 21 family members which are responsible for the formation and isomerisation of disulphide bonds in proteins. PDI possesses both chaperone and disulphide interchange activity, but it is unclear which property is important in protecting against mutant SOD1-induced toxicity. The aim of this study was to investigate the mechanism of action of PDI and to determine whether other PDI family members are protective in ALS. **Methods** Neuronal cell lines were co-expressed with mutant SOD1 and either wild-type PDI, or a PDI mutant in which all four cysteine residues necessary for disulphide interchange activity were mutated to serine. Similarly, mutant SOD1 was co-expressed with either ERp57, PDIA2 (PDIp) or ERp72. Inclusion formation, ER stress and apoptosis were examined in these cell lines. **Results** PDI mutant was unable to protect against mutant SOD1-induced inclusion formation, ER stress and apoptosis, revealing that the disulphide interchange activity of PDI is necessary for this function. ERp57 was equally protective as PDI against mutant SOD1-induced pathologies; however ERp72 and PDIA2 had no effect. **Conclusion** The disulphide interchange activity as opposed to the chaperone activity of PDI is important in its protective function in ALS. However there is also substrate specificities underlying this activity because not all PDI family members are protective, despite the presence of the same active site (CXXC).

POS-TUE-177

VACCINATION FOR NEUROPATHIC PAIN FOLLOWING PERIPHERAL NERVE INJURY

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Purpose: Neuropathic pain (NP) occurs as a result of a lesion, disease or trauma to the somatosensory nervous system, adversely affecting the quality of health and social lives of sufferers. Mounting evidence suggests that pro-inflammatory T cells play a vital role in the development and maintenance of NP. Altered peptide ligands (APLs) are mutant peptides that can significantly modulate the T cell immune response. Therefore, we assessed the effects of immunisation with a myelin-derived APL on pain hypersensitivity in an animal model of NP following peripheral nerve injury. **Methods:** Lewis rats were vaccinated subcutaneously with either a weakly encephalitogenic peptide of myelin basic protein (MBP) (cyclo-MBP_{87-99}, 250µg, n=6), APL (cyclo(87-99)[A^{91},A^{96}]MBP_{87-99}, 250µg, n=6) in Complete Freund's Adjuvant (CFA) (1mg/ml) or CFA only (control, 1mg/ml, n=6), following chronic constriction injury (CCI) of the left sciatic nerve. Pain hypersensitivity was tested by measurements of paw withdrawal threshold to mechanical stimuli, regulatory T cells were analysed by flow cytometry (n=4, days 10 and 30 post-CCI), and immune cell infiltration was assessed by immunohistochemistry (n=3, day 30 post-CCI). **Results:** Nerve-injured rats immunised with APL showed significantly less pain hypersensitivity compared to rats immunised with cyclo-MBP_{87-99} and CFA only on days 8, 10 ($P<0.01$),19 and 23 ($P<0.05$) post-CCI. Furthermore, T cell numbers were significantly lower ($P<0.01$) in the injured nerve in rats immunised with APL as compared to cyclo-MBP_{87-99} injected rats. However, there was no significant difference in the prevalence of systemic regulatory T cells among the three groups. **Conclusion:** These results suggest that immune deviation by active immunisation with a non-encephalitogenic myelin-derived APL mediates an analgesic effect in neuropathic animals.

POS-TUE-178

UNCORRECTED ANTISACCADE ERRORS PREDICT COGNITIVE PROBLEMS AFTER PAEDIATRIC MILD TRAUMATIC BRAIN INJURY

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Purpose: To determine whether performance on saccadic eye movement tasks could be predictive of ongoing cognitive difficulties following paediatric mild traumatic brain injury (mTBI). **Method:** 45 mTBI patients and 44 age-matched controls (aged 8-15) were tested over three sessions, at first contact (within 2 weeks of injury for mTBI participants), and 3 and 6 months following the first session. Participants completed a battery of eye movement tasks including prosaccade, antisaccade and self-paced saccade tasks. Cognitive testing was undertaken with the ImPACT concussion assessment software, evaluating attention, verbal and visual memory, processing speed and reaction time. Prosaccade and antisaccade latency, gain and peak velocity were assessed, as were corrected and uncorrected antisaccade errors, and self-paced saccade rate/30 s. Saccadic and cognitive measures were compared between groups. **Results:** Significant differences between groups at time 1 were not found for most saccade measures. However, outlier identification on box plots of uncorrected errors on the antisaccade task revealed that at time 1 all of the younger mTBI children (aged 8-9) comprised the highest outliers. Analyses were therefore conducted comparing the young mTBI group (N=5) with age-matched controls (N=10) on the eye movement and cognitive measures. The results revealed that at time 1 the young mTBI group made significantly more uncorrected errors on the antisaccade task, and had significantly longer correction latencies when correcting antisaccade errors. Results of the cognitive testing, particularly verbal and spatial memory, revealed increased processing speeds and poorer working memory capacity of mTBI participants at time 2 and 3, respectively. This was not true of the mTBI population as a whole. **Conclusions:** Young children may be at risk of subtle, persistent cognitive sequelae from even mTBI.

POS-TUE-179

RESTING-STATE FUNCTIONAL CONNECTIVITY IN HUNTINGTON'S DISEASE: THE IMAGE-HD STUDY

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Purpose: Functional and structural neural impairments have been documented in both symptomatic Huntington's disease (symp-HD) and premanifest gene carrier (pre-HD) individuals. The aim of this study was to characterize resting state connectivity in both pre-HD and symp-HD individuals. **Methods:** fMRI data was acquired via a 3T MRI from 25 pre-HD, 23 symp-HD, and 18 healthy controls. The fMRI data were pre-processed and analyzed using a data-driven method to identify several resting state networks. Voxel-wise synchronization of networks of interest were compared between groups using dual-regression and statistically tested using a permutation based non-parametric method. **Results:** Nine well-established resting state networks were identified. Of the nine networks, four were significantly altered in both the pre-HD and symp-HD groups. Compared with controls, pre-HD individuals showed decreased synchrony in the sensorimotor (primary motor cortex) and dorsal attention (visual cortex) networks. Compared with controls, the symp-HD individuals showed widespread reduction in synchrony in the dorsal attention network. There was also a functional disconnection of the posterior putamen and superior parietal cortex from the frontal executive network in the symp-HD, compared with control and pre-HD individuals. Furthermore, the left fronto-parietal network showed areas of increased synchrony in symp-HD, compared with pre-HD individuals. **Conclusion:** We speculate that reduced synchrony in both the sensorimotor and dorsal attention networks may serve as an early signature of neural change in pre-HD. The widespread reduction in dorsal attention and cortico-striatal networks may contribute to the development of clinical symptoms in HD.

POS-TUE-180

TESTOSTERONE REGULATION OF SEX STEROID SENSITIVITY ALONG THE NIGROSTRIATAL PATHWAY IN ADOLESCENT MALE RATS

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Peak age of onset of schizophrenia in males overlaps with adolescent increases in testosterone, implicating testosterone in the precipitation of dopamine-related psychopathology. Increased dopamine activity in the dorsal striatum is a driver of schizophrenia-related psychosis. MRI studies report sexual dimorphism of caudate and putamen. The analogous area in songbirds, the higher vocal centre, is larger during breeding season, correlating with higher testosterone. Testosterone exerts effects via androgen receptor (AR) directly or after 5α-reduction to dihydrotestosterone (DHT), and after aromatisation to 17β-estradiol (E2), via estrogen receptors (ER). Purpose: To determine if and how testosterone modulates sex steroid receptor and steroidogenesis-related mRNAs in the adolescent male rat substantia nigra (SN) and caudate putamen (dorsal striatum). Methods: Pre-adolescent rats (45-days old; ~14/group) underwent sham gonadectomy or were gonadectomised and given 2-week testosterone, E2 or DHT replacement. Sex steroid receptor, 5α-reductase and aromatase mRNAs were measured in SN and dorsal striatum. Results: In SN, AR mRNA was increased by all sex steroids and ERβ increased by androgens. ERα mRNA was decreased by DHT. In striatum, there were no significant changes in receptor gene expression after gonadectomy or with replacement. 5α-reductase mRNA was increased by DHT in the SN but unchanged by sex steroids in striatum. In both regions, aromatase mRNA was decreased by gonadectomy but was unchanged by replacement. Conclusion: Striatum was less sex steroid sensitive than SN with respect to sex steroid receptor and 5α-reductase mRNA in adolescent male rats. Testosterone regulation of sex steroid sensitivity in the nigrostriatal pathway involves regulation of gene expression in the region of dopaminergic soma rather than the region of the striatal targets.

POS-TUE-181

THE TUMOR SUPPRESSOR PTEN IS TRANSPORTED IN EXOSOMES FOR PHOSPHATASE ACTIVITY IN RECIPIENT CELLS

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Purpose: Gliablastoma multiforme is the most common and most malignant form of glia tumours. PTEN (phosphatase and tensin homolog), a tumour suppressor is non-functional and mutated in about 60% of Glioblastoma tumours. Here, we describe a new way to introduce functional PTEN back into Glioblastoma cells by using exosomes as a delivery tool. Exosomes, small secreted vesicles, are intercellular messengers with the capacity to alter the internal physiological states of recipient cells. **Methods:** We used supernatant from wild type and Ndfip1 KO MEFs to harvest exosomes for electron microscopy, western blotting and uptake experiments. All experiments were done at least three times. **Results:** We demonstrate that PTEN, a tumor suppressor protein normally localized in the cytoplasm and nucleus, can be secreted in exosomes. Secreted PTEN can be internalized by recipient cells with resultant functional activity, exhibited by reduced pAkt and recipient cell proliferation. PTEN secretion in exosomes requires Ndfip1, an adaptor for Nedd4-family ubiquitin ligases, and absence of Ndfip1 abolishes exosomal trafficking of PTEN. These results identify Ndfip1, a key member of the Nedd4 ubiquitination pathway, to be an important molecular regulator for exosomal export of PTEN, with consequences for non-cell autonomous PTEN activity. **Conclusion:** The ability of PTEN to exert phosphatase activity in recipient cells has significant implications for PTEN function during development, health and disease.

POS-TUE-182

FUNCTIONAL ADULT HIPPOCAMPAL NEUROGENESIS IN R6/1 HUNTINGTON'S DISEASE MICE

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Purpose: Huntington's disease (HD) is a fatal neurodegenerative disorder affecting a range of cellular functions in the brain, including deficits in adult hippocampal neurogenesis (AHN). We tested the effects of sequential voluntary running followed by environmental enrichment on AHN and examine evidence of functional AHN in female R6/1 HD and wildtype littermate mice. **Methods:** Basal (standard-housed) and exercise-induced (7-days of wheel running) hippocampal precursor proliferation was quantified 1 day after BrdU administration by Ki67 and BrdU immunohistochemistry. Functional AHN was quantified 6 weeks after BrdU administration in standard-housed and running (7 days) then enriched-housed (7 days) mice. Basal and exercise-induced (2 hours of wheel running) serum growth hormone (GH) and insulin like growth factor-1 (IGF-1) concentrations were determined by ELISA. Basal and exercise-induced (7 days of wheel running) hippocampal protein levels of IGF-1 receptor, and total and phosphorylated Akt were quantified by Western blot. **Results:** R6/1 mice consistently ran significantly lower distances. Sequential running then enrichment induced a 3-fold increase in AHN in wildtype but not R6/1 HD mice. Both genotypes displayed indirect evidence of functional AHN through cFos/NeuN/BrdU triple labeling. Running increased serum GH without change in serum IGF-1 in both genotypes. In the hippocampus; IGF-1 receptor levels were unchanged, basal and exercise-induced total Akt was reduced in R6/1 mice and running increased Akt phosphorylation in wildtype but not R6/1 mice. **Conclusions:** The sequential combining of running followed by enrichment did not rescue AHN deficits in female R6/1 mice. However, we found indirect evidence of functional AHN in R6/1 female mice. Reduced running distances and reduced Akt phosporylation could underlie the failure of exercise-induced AHN in female R6/1 mice.

POS-TUE-183

SYMPATHOLYTICS ATTENUATE CARDIAC AND CORTICAL ELECTROGRAPHICAL CHANGES DURING STATUS EPILEPTICUS

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Purpose: Status epilepticus (SE) has been increasingly associated with cardiac injury in clinical and animal studies. The current study examined the effect of kainic acid (KA, 10 mg/kg) induced seizures on EEG and ECG activity. It was hypothesised that atenolol, a peripheral β2 antagonist, and clonidine, a α2 agonist, would attenuate SE-induced cardiac arrhythmias and structural damage. Methods: Sprague-Dawley rats (male, 300-350g) were implanted with EEG and ECG electrodes to allow simultaneous telemetric recordings of CNS cortical and cardiac responses during and after seizures. Animals were randomised into saline-controls, and saline-, atenolol (5 mg/kg)- and clonidine (0.1 mg/kg)- pretreated KA groups (n=7-8 per group). Results: Bradycardia, with decreased P wave amplitude coinciding with low level seizure activity, was observed within the immediate period following KA administration. Heart rate decreased maximally by 27.6 ± 5.9% in the saline-KA group. As high level seizure behaviours and EEG spiking progressively increased, tachycardia developed, with a maximum heart rate increase of 33.1 ± 7.4 % coinciding with QTc prolongation and T wave elevation over the remainder of the 3 hour recording period. Maximal increases in EEG spiking occurred across all frequency bands (delta-gamma) were recorded 125 min post-KA. Pretreatment with atenolol and clonidine reduced KA-induced changes in heart rate, QTc interval and T wave amplitude observed during both bradycardic and tachycardic phases. Pre-administration of both atenolol and clonidine also successfully reduced seizure activity across all frequency bands and decreased seizure behaviours. Conclusion: These results suggest that the modulation of sympathetic activity either systemically by atenolol or centrally by clonidine during SE provides a promising therapeutic approach to seizure-induced cardiomyopathy as well as decreasing seizure severity.

POS-TUE-184

SEXUALLY DIMORPHIC DOPAMINERGIC DYSFUNCTION IN A TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE AND DEPRESSION

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Purpose: Depression is the most common psychiatric disorder in Huntington's disease (HD) patients. There is yet to be a systematic study of sexual dimorphism in the development and presentation of depression in HD patients, although it is known that depression in the general population is more common in women. We have previously reported a depression-like phenotype in the R6/1 transgenic mouse model of HD associated with serotonergic system alterations. We now extend these findings to include sexually dimorphic dopaminergic (DA) dysfunction at an early pre-motor symptomatic disease stage. Methods: In order to investigate whether transgenic HD mice display depressive-like endophenotypes associated with dopaminergic impairments, we assessed the effect of several dopaminergic ligands (including the DA transporter inhibitor bupropion and the D1 receptor agonist SKF-81297) on the forced-swim test (FST) and on locomotor activity in male and female R6/1 HD mice at 8-12 weeks of age. Results: We found that compared to female animals, males were more sensitive to the locomotor stimulating effects of bupropion (which were successfully attenuated with the selective D1 antagonist SCH-23390). In addition, 8-week-old HD females (but not males) showed an impaired locomotor response to bupropion. The HD mutation also resulted in a decrease of locomotor response to the D1 agonist SKF-81297. Finally, the depressive-like behavior exhibited by female HD mice in the FST was rescued by acute bupropion, possibly through a mechanism involving D2/D3 receptor signaling. Conclusion: Our data suggest a crucial role for disrupted dopaminergic signaling in mediating the sexually dimorphic depression-like phenotype in HD mice and provide evidence suggesting that bupropion could be explored as a potential antidepressant in HD.

POS-TUE-185

EVOLUTION OF ISCHEMIC DAMAGE OVER 6 MONTHS AFTER STROKE IN THE RAT

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Purpose: Infarct volume is the most common outcome of experimental stroke studies, yet is often only assessed acutely. This study aims to document the development of histological damage over 6 months post stroke. **Methods:** 132 Spontaneously Hypertensive Rats underwent thread occlusion MCAo for 90 minutes or sham, with stroke animals randomised to recovery time: 1, 3, 7, 14, 21, 28 days, 12 and 24 weeks (n≥11 per group). Damage was delineated on H&E stained sections. **Results:** 90 minute MCAo resulted in a medium sized cortical and striatal infarct. Acute damage was characterised by infarction of the striatum and cortex, with oedema peaking at 12.7% at 3 days. Oedema resolved by 7 days. Atrophy of the ipsilateral hemisphere was evident from 28 days. Macrophages and other infiltrating cells packed the area of infarct from 7 days, facilitating clearance of damaged tissue to leave a fluid filled cavity, which grew from 14 days. Whilst the volume of damage changed over time, an equivalent proportion of tissue was lost at all time points (26±7-34±12% of contralateral hemisphere). **Conclusion:** Damage progressed from a necrotic infarct to a fluid filled cyst over time, with hemispheric size changing in relation to the type of damage. Variability in the volume of damage may be due to individual differences in the rate of clean up of the infarct. Examining the development of behavioural and histological damage to chronic timepoints is an important step in both understanding stroke and bringing animal models closer to the clinical situation.

POS-TUE-186

THE EFFECT OF CHRONIC METFORMIN TREATMENT ON RETINAL FUNCTION AFTER PRESSURE INJURY IN AGED MICE

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Purpose: Metformin is an extensively used anti-diabetic agent; beyond its hypoglycemic effect it possesses some neuroprotective properties as well. Metformin mechanisms of action are not fully understood, but it is acknowledged that AMPK activation is required for many of metformin effects. The AMP-activated protein kinase (AMPK) proposed to play a central role in various neuroprotective interventions. The aim of this study is to verify whether metformin could decrease retinal ganglion cell (RGC) age-related vulnerability to pressure-induced injury. **Methods:** C57Bl/6 mice at an age of 18 months were administered with 300mg/kg metformin in drinking water for 6 weeks. Pressure injury involved an acute elevation of intraocular pressure (IOP) through cannulating the anterior chamber of the. Retinal function was assessed using electroretinography (ERG) before and after IOP injury in young (3 months) (n=9), old (18 months) (n=10), and metformin-treated (18 months) (n=9) mice. **Results:** In response to pressure-induced injury old mice showed a 45% reduction in inner retinal function, which arises primarily from RGCs. In contrast young mice showed only a slight reduction (about 15%) in inner retinal response following IOP elevation. No significant difference was found between metformin-treated and control 18 months groups across all components of ERG in response to injury. **Conclusion:** Our results suggest that chronic metformin treatment in drinking water is not able to inhibit age-related increased susceptibility of retinal ganglion cells to elevated IOP injury. Future experiments will consist of improving the bioavailability of the drug using a parenteral route of administration along with using a more potent AMPK activator such as AICAR.

POS-TUE-187

LONG-TERM INTERMITTENT HYPOXIA ELEVATES COBALT LEVELS IN THE BRAIN AND INJURES MYELIN

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Purpose: Exposure to the variable oxygenation patterns in obstructive sleep apnea (OSA) causes oxidative stress within the brain. We hypothesized that this stress is associated with an increase in the levels of redox-active metals. **Methods:** To model OSA, adult male C57BL/6J mice were exposed to long-term intermittent hypoxia (LTIH; n = 20) for 10 h / d for 8 weeks or sham-LTIH (normoxia control condition; n = 21). **Results:** Laser ablation-inductively coupled plasma-mass spectrometry was used to quantitatively map the distribution of the trace elements cobalt, copper, iron and zinc in forebrain sections. Control mice contained 62 ± 7 ng cobalt/g wet weight, whereas LTIH mice contained 5600 ± 600 ng cobalt/g wet weight ($p \leq 0.0001$). Other elements were unchanged between conditions. Cobalt was concentrated within white matter regions of the brain, including the corpus callosum. Ultrastructural examination of the corpus callosum revealed disorganized myelin sheaths ($P \leq 0.001$) and degenerated axon profiles ($p \leq 0.05$) in LTIH mice. **Conclusion:** The brain levels of cobalt (but not of other metals) are elevated in response to intermittent hypoxia, particularly in the cerebral white matter. Since elemental cobalt is neurotoxic, the abnormally high levels of cobalt may contribute to the oxidative stress and dysmyelination that occur in LTIH. Alternatively, the higher levels of cobalt may indicate that vitamin B_{12} (cobalamin) is sequestered in white matter in order to repair and stabilize myelin.

POS-TUE-188

INTEGRATION OF MRNA AND MICRORNA EXPRESSION PROFILES IN OPTIC NERVE CRUSH MODELS

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It has long been accepted that insight into the molecular requirements for successful nerve regeneration can be gained from studying species in which the central nervous system (CNS) spontaneously regenerates, such as in zebrafish. This contrasts with the weak response displayed by mammals (e.g. rats), which ultimately results in cell degeneration and dysfunction. Previous microarray studies that examined the robust regenerative response of zebrafish after an optic nerve injury (a common model used to investigate CNS regeneration) have failed to find a single gene or underlying genetic mechanism responsible for this difference. Thus it is likely that the altered regulation of signaling pathways, involving key genes, contributes to the high level of neuronal survival and axonal regeneration observed in zebrafish. One such regulatory mechanism that influences post-transcriptional gene expression is microRNA expression. In order to delineate the role of microRNAs in regulating successful nerve repair, we have undertaken an integrated profiling study to characterise gene expression and regulation in both zebrafish and rat retinal tissue following an optic nerve crush injury. Retinal tissue from both species was collected and processed for mRNA profiling on Agilent 4x44K microarray chips and Exiqon LNA microRNA arrays. **Preliminary results** highlight the complexity of this regulation, with qPCR data showing a decrease in post-injury expression of miR-124 in both species, but with conflicting changes in the expression level of predicted downstream target genes, i.e., decreased miR-124 expression correlated with an up-regulation of cytoskeletal-associated genes ARHGDIA, ARPC1B and VIM in rat tissue, in contrast to the down-regulation of the same genes in zebrafish retinae. By performing a comparative bioinformatic analysis that integrates mRNA and miRNA data, we anticipate that intra and inter-species comparisons will enable identification of critical signaling pathways involved in nerve repair, and the specific microRNAs that regulate these pathways.

POS-TUE-189

AMYLOID BETA1-42 UP-REGULATES EXPRESSION OF SORTILIN MRNA AND PROTEIN IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Purpose: Sortilin is a Golgi sorting protein that belongs to VPS10P family which mediates amyloid precursor protein (APP) trafficking in neurons. Sortilin interacts with BACE1 and regulates APP processing. It has been reported that Sortilin expression increases in post-mortem brain of Alzheimers disease (AD) patients. In the present study, we examined if Amyloid beta regulates the expression of Sortilin mRNA and protein in human neuroblastoma cells. **Method:** SH-SY5Y cells were treated with different concentrations of Amyloid beta1-42 oligomer (5, 10, and 20 μM) for different time courses and then the cell lysate was subjected to Western blots for quantification of Sortilin and APP proteins and Real-Time PCR for the quantification of mRNA levels of Sortilin, SorLA, APP, and BACE1. **Results:** The results show that Sortilin gene and protein expressions were significantly up-regulated respectively after 4 and 24 hours treatment with 5 μM Aβ1-42 in SH-SY5Y cells (n=6) ($p \leq 0.05$). Treatment with 5 μM Amyloid beta1-42 for 24 hours enhanced APP mRNA level (n=6) ($p \leq 0.05$), but had no effect on APP protein expression (n=6). We also found that SorLA and BACE1 mRNA levels were slightly elevated after 12 and 24 hours treatment with 5 μM Aβ1-42. **Conclusion**: In conclusion, this study indicates that Amyloid beta1-42 oligomer increases Sortilin expression in SH-SY5Y human neuroblastoma cells and suggesting a potential physiological interaction of Amyloid beta and Sortilin in Alzheimers disease. Keywords: Amyloid beta, Sortilin, APP, Alzheimers disease.

POS-TUE-190

GENE-MICRORNA INTERACTIONS ASSOCIATED WITH ANTIPSYCHOTIC MECHANISMS AND THE METABOLIC SIDE EFFECTS OF OLANZAPINE

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Purpose: Antipsychotic drugs (APDs) have been shown to induce changes in gene expression in the brain. We sought to investigate whether microRNA expression is also altered and what functional implications such changes may have. We also investigated the possible functional interplay of miRNA-gene regulatory interactions. **Methods:** 76 C57BL/6 mice were treated with haloperidol, olanzapine, clozapine or saline for 7 days. High throughput miRNA profiling of RNA extracted from whole brain was performed and gene target predictions of miRNA conducted for functional analysis. Gene expression data was integrated and miRNA-gene regulatory interactions identified using the Bayesian Networks with Splitting-Average method. **Results:** Six miRNA were significantly altered with haloperidol treatment and five with olanzapine or clozapine treatment (FDR<5%), with three of these validated by Q-PCR ($p<0.05$). These miRNA have putative schizophrenia candidate gene targets and potential neurologically relevant influences. Metabolic pathways and functions, such as weight gain, were enriched in the treatment with atypical APDs. Significant gene-miRNA interaction networks were identified in the olanzapine group with neurological and metabolic relevance. **Conclusion:** This study is the first to suggest a role for miRNA in the mechanism of antipsychotic action and the metabolic side effects of the atypical APDs, and thus supports the importance of miRNA in pharmacogenomics.

POS-TUE-191

POST-SURGICAL ALLODYNIA AND HYPERALGESIA

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Purpose: In most cases following a surgical procedure pain is experienced at the site of the surgery, however patients may also report painful sensations in sites adjacent to the procedure. Our aim in this study was to characterise the motor and sensory perturbations that arise following peripheral nerve damage. **Methods:** All surgery was conducted under anaesthesia (isoflurane 2-5% in 100%0_2) in Long Evan rats. In nerve section experiments (n=60) the left median nerve was transsected, repaired (using a photochemical bonding procedure) and allowed to recover. In sham experiments (n=20) identical surgery was performed, however the median nerve was left intact (sham). In both cases identical procedures were used to close the wound. Assessments of motor function (grip force) and sensory function (tactile, warm, cool and noxious heat) were made for up to 90 days following surgery. **Results:** One week following nerve section, animals were unable to grasp with the left paw, and grip strength was significantly reduced in the right paw. Similar effects were observed in the sham animals in which the median nerve was intact. Sensory testing in the distal paws revealed significant reductions in withdrawal latencies for mechanical and noxious heating observed in both the nerve section and sham groups. A pronounced intolerance to cooling (12°C) emerged in both groups that was not observed prior to the surgery. Conclusion: Our results suggest that the surgery in the forelimb to expose the median nerve is sufficient to produce a generalised hypersensitivity that extends distally into both the ipsilateral and contralateral forepaws such that normally innocuous stimuli are perceived as painful.

POS-TUE-192

UNDERSTANDING NEUROANATOMICAL AND NEUROSTRUCTURAL CONTRIBUTIONS TO RELATIVE SEIZURE DISPOSITION AND ASSOCIATED BEHAVIOURAL PROFILES: LESSONS FROM FAST AND SLOW RATS

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Purpose: Epilepsy and Autism Spectrum Disorder (ASD) often share both primary and comorbid symptoms. The comorbid symptoms associated with these neurological disorders include seizures, developmental delay, hyperactivity, impulsivity, aggression and cognitive impairments. The degree of clinical overlap is believed to signify a 'spectrum of vulnerability' that arises out of an early common dysfunction in Central Nervous System (CNS) development. This can be investigated using seizure-prone (FAST) and seizure-resistant (SLOW) rats strains developed via selective breeding processes based on a differential susceptibility to amygdala kindling. Remarkably, along with seizure susceptibility, the FAST strain has additional traits naturally evolved that are highly reminiscent of those observed in ASD. Therefore, neuroanatomical and neurostructural alterations in FAST versus SLOW rat strains may reveal the common CNS dysfunction associated with these two interrelated disorders. **Methods:** We studied neuroanatomical discrepancies in FAST versus SLOW rats using magnetic resonance imaging (MRI). With known white matter alterations reported in both epilpesy and ASD we also compared levels of two primary myelin proteins in FAST versus SLOW rats using western analysis. **Results:** MRI study revealed that FAST (n=14) rats versus SLOW (n=9) rats, have significatnly larger volume in white matter including corpus callosum and superior posterior cerebellum; hippocampus and total ventricles. In addition, the levels of MBP and PLP were found to be significantly reduced in FAST (n=3) versus SLOW (n=3) myelin. **Conclusion:** The enlarged white matter volumes and altered myelin microstructure observed in FAST versus SLOW rats may be related to the profound developmental delay, abnormal behavioral patterns and heightened seizure susceptibility in FAST versus SLOW rats.

POS-TUE-193

RESILIENCE VS GENOTYPE AND PAST/RECENT STRESSORS AS PREDICTORS OF DEPRESSION

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Purpose: The short form of the serotonin transporter gene 5-HTTLPR has a robust overall association with depression following stress. However, nearly 40% of studies reviewed in a recent meta-analysis reported results that did not support that hypothesis, suggesting the presence of intervening variables in that gene x environment relationship. Further, the great majority of studies on this association have used patients suffering from Major Depressive Disorder (MDD), and few have examined community samples. Therefore, the current study investigated the 5-HTTLPR and previous and current stress, and compared the interaction of those variables with psychological resilience (previously found to act as a 'buffer' against depression) for their relative power to predict depression in a community sample. Methods: 67 adult female and 59 adult male volunteers gave a mouthwash sample for genotyping, and also completed scales assessing childhood stressors, recent stressors, resilience and depression. Results: None of genotype, childhood or recent stressors was significantly associated with depression scores, but resilience was a significant inverse predictor of depression scores and also the presence of clinically significant depression. Conclusions: These data add to several previous reports in failing to show a significant association between the short form of the 5-HTTLPR and depression. By contrast, the role of psychological resilience as a strong inverse predictor of depression was confirmed with this community sample. These results suggest that the gene x environment interaction hypothesis might be strengthened by inclusion of resilience as an indicator of an intervening variable between stress, genes and depression.

POS-TUE-194

DISTURBANCES IN THE ENDOSOME-LYSOSOME SYSTEM IN SPINAL CORDS OF ALS PATIENTS

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Purpose: The mislocalisation and aggregation of misfolded or mutant ALS-linked proteins are common hallmarks of familial and sporadic ALS pathology. This evidence, as well as the early involvement of endoplasmic reticulum stress and autophagy, suggests disruption of intracellular vesicle trafficking contributes to motor neuron injury and death. Endosomes are the major transport vesicles in neurons and evidence linking mutations of ALS2, CHMP2B and FIG4 to ALS support the role of the endosome-lysosome system (ELS) in ALS. We investigated whether key ELS markers are differentially expressed in ALS. **Methods:** Expression of ELS markers in spinal cords from post mortem tissue of control (n=5), familial (n=3) and sporadic (n=5) ALS patients were examined using western blotting and immunohistochemistry. **Results:** ALS spinal cords had increased expression of the endosomal markers Rab5 (1.5 fold, $P<0.05$) and Rab 11 (~3 fold, $P<0.05$), when compared to controls, while no changes in the lysosomal markers LAMP2A and cathepsin D were observed. Increased expression of the autophagy marker p62 (~2 fold, $P<0.05$) and the chaperone Hsc70 (~2 fold, $P<0.05$) were also found in ALS spinal cords, together with increased expression of 20S proteasome (~3 fold, $P<0.01$). **Conclusion:** Our results demonstrate that a number of key players in the ELS are upregulated in spinal cords of ALS patients. The increases in p62 and 20S proteasome also indicate the importance of protein degradation in ALS pathology. These data give support for a vital role of the ELS in ALS pathology and provide possible targets for therapeutic intervention.

POS-TUE-195

MITOCHONDRIAL CONTRIBUTIONS TO NEURONAL PROGRAMMED CELL DEATH: EVIDENCE FOR AN INTERFACE OF AUTOPHAGY WITH MITOCHONDRIAL ENERGETICS

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Involvement of dysfunctional mitochondria is recognized as a common theme amongst various neuropathologies. As key regulators of cell death, their influences on programmed cell death (PCD e.g. apoptosis, autophagy) determine differential death outcomes of neurons. **Purpose:** To investigate the role of mitochondrial respiratory chain complexes in recruitment of autophagy and to evaluate the interface of autophagy with mitochondrial energetics. **Methods:** Primary cultures of cerebellar granule cells (CGCs; Swiss mice) were exposed to insults targeting mitochondrial respiratory chain complexes I-IV (rotenone, 3-nitropropionic acid, antimycin A and KCN, respectively) and drugs that induce PCD: staurosporine (STS; apoptosis) and H_2O_2 (oxidative stressor). ATP content was determined by bioluminescent detection of light using luciferin. Western immunoblotting and cytochemistry techniques were performed to observe specific autophagic markers. **Results:** All stressors produced mitochondrial dysfunction as shown by reduction in MTT activity ($n\geq3$). Inhibition of mitochondrial respiratory complexes induced puncta formation of microtubule-associated protein 1 light chain 3 (LC3-I/II), and puncta staining of an autophagic vacuole marker, monodansylcadaverine, further supported the induction of autophagy under these conditions. The concentration-, time-dependent enhancement of LC3-II bands by immunoblotting ($n\geq3$) was interrelated to the concentration-, time-dependent decreases of ATP level under mitochondrial respiratory chain complex inhibition, STS and H_2O_2 treatment ($n\geq3$). This evidence suggests the involvement of respiratory complexes in the recruitment of autophagic mechanisms. **Conclusion:** These data propose an interface between recruitment of autophagy and mitochondrial energetics. Autophagic mechanisms are recruited to PCD by diverse cellular insults including those mediated via respiratory complexes.

POS-TUE-196

REGULATION OF PP2A METHYLATION BY GENETIC/ DRUG/DIETARY INTERACTIONS - IMPLICATION FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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Altered folate status has been implicated in a number of age-related neurodegenerative disorders including Alzheimer disease (AD) and Parkinson's disease (PD). Folate metabolism and the methylation of Ser/Thr protein phosphatase 2A (PP2A) are intimately related. We have previously reported in cultured cells and *in vivo* that folate deficiency induces the loss of methylated PP2A/Bα holoenzymes, concomitant accumulation of demethylated PP2A, and enhanced phosphorylation of tau, a key neuropathological marker of AD. **Results**: Here we show that, relative to controls, PP2A methylation status is altered in the brain of aged $MTHFR^{+/-}$ mice (n=6) that reproduce biochemical/ clinical consequences of MTHFR C677T polymorphisms in human. Moreover, acute adminsitration of L-dopa, a drug routinely used to treat PD patients, causes down regulation of methylated PP2A enzymes and increased phosphorylation of tau in wild-type mice (n= 6). These effects are exacerbated by folate deficiency. **Conclusion:** Our findings unveil methylation-dependent mechanisms by which dietary folate and L-Dopa, as well as common folate gene polymorphisms can interact to affect the regulation of PP2A and tau, with potential detrimental effects to neuronal cells.

POS-TUE-197

MOTOR NEURON EXCITOTOXICITY IS AFFECTED BY GLIAL CELLS

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Purpose: Excitotoxicity in motor neurons and surrounding cells has been implicated as a major contributer to pathology in amyotrophic lateral sclerosis. Specifically, excitotoxic conditions may alter the normal communications between motor neurons and glial cells and trigger a degenerative environment. Increasing evidence suggests that excitotoxicity is not restricted to the neuronal cell body and that the axon may be a primary target for excitotoxicity. Additionally, the non-neuronal cells of the lower motor neuron circuit may be key in modulating this effect in a site-specific manner. **Methods:** Primary spinal motor neurons were plated onto laminin coated coverslips or onto glial cells and treated at 21 days *in vitro* with 25μM kainic acid (n=3 separate cultures). Motor neurons were fixed at 6 hours post treatment, immunolabelled with β-III-tubulin and surviving motor neurons counted. Cells were counted in $3mm^2$. **Results:** Motor neurons co-cultured with glial cells had significantly ($p<0.05$) fewer motor neurons surviving (17.3±1.33) compared with untreated controls (29.0±4.12). Motor neurons cultured on laminin alone did not have a significant ($p>0.05$) decrease in number of motor neurons from untreated controls at 6 hours (36.8±5.73 treated, 33.0±9.50 control). Preliminary data in compartmented culture indicates glial cell-mediated toxicity may indeed act in a site-specific manner. **Conclusions:** This result is likely due to the effect of monolayer culture with motor neuron distal axons in contact with the glial cells as opposed to muscle cells as occurs *in vivo*. This data highlights the crucial nature of appropriate cell organisation within culture models.

POS-TUE-198

NEUROMODULATION USING TRANSABDOMINAL ELECTRICAL STIMULATION TREATS PAEDIATRIC CHRONIC SLOW-TRANSIT CONSTIPATION

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Purpose: Colonic motility and defecation are controlled by enteric nerves and the central nervous system via the vagal and pelvic nerves. Direct stimulation of sacral nerve S3 increases colonic motility and overcomes slow-transit constipation (STC). Electrical neuromodulation can also be applied across the skin. In 2005-09, we showed that 12 sessions of transcutaneous electrical stimulation (TES) using interferential current (IFC) delivered in physiotherapists' clinics, (20 mins/session, 3 times/week) increased colonic motility. Aim: Determine the effects of 3-6 months of daily TES-IFC delivered at home on defecation and soiling in children with STC. Methods: Parents were trained to administer TES at home. TES-IFC was administered on top of existing laxative treatment. Four electrodes (4cm x 4cm) were placed, 2 on the belly and 2 on the back with currents crossed. Sixty-two STC children (28 male; 2-16yrs, mean 7yrs, diagnosed by radio-nuclear transit study) had stimulation with IFC (4000Hz carrier frequency, 80-160 Hz beat frequency) for 1 hr/day. Defecation, soiling and laxative use were recorded daily before and during treatment. Results: Defecation frequency increased in 54/56 children who started with <3 defecations/wk (mean±SEM 1.43±0.6 pre to 4.0±1.5 episodes/wk, $p<0.0001$) with 32/56 increasing to >3 defecations/wk. Urge-initiated defecation increased in 54/62. 37/39 (95%) who had abdominal pain reduced pain (2.7±1.6 to 0.4±0.6 episodes/wk, $p<0.0001$). Soiling decreased in 54/62 (87%) from 5.3±1.7.4 to 1.1±1.5 episodes/wk, $p<0.0001$. Good clinician training and close patient contact were needed. Conclusion: Non-invasive transabdominal neuromodulation administered at home increased defecation and reduced soiling in STC children. Further studies are required to determine which nerves are affected.

POS-TUE-199

DIRECT BACTERIAL ENTRY INTO THE BRAIN: *BURKHOLDERIA PSUEDOMALLEI* AND THE OLFACTORY NERVE

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Melioidosis is a potentially fatal disease endemic to northern Australia which is caused by the bacteria *Burkholderia pseudomallei*. In the Northern Territory in 2009/2010, the disease had an incidence of 50-100/100,000*. There is a mortality rate of around 14%. Purpose: It is unclear how the bacteria penetrate the brain and cause the neurological form of the disease. One potential route is via the olfactory and trigeminal nerves of the nasal cavity. Methods: We inoculated mice with *B. pseudomallei* for 24-48 hrs (n=6 animals at each timepoint) and analysed them for localisation of the bacteria within the nasal cavity. Results: Two levels of infection occurred. In widespread major infection, the olfactory epithelium rapidly responded by degradation and an immune response which limited the penetration of bacteria in the mucosal layer. In contrast, in low level minor infection, very small numbers of bacteria penetrated the olfactory mucosa without causing degradation of the epithelium or an obvious immune response. In both levels of infection, the bacteria penetrated and colonised the olfactory and trigeminal nerves and migrated directly into the olfactory bulb within central nervous system. Importantly, we have previously determined that the cells of the immune system, macrophages, are largely excluded from olfactory nerve bundles. We instead propose that the olfactory glia are the primary cells responsible for the phagocytosis of bacteria within the olfactory nerve and act to limit the spread of infection. Conclusion: These results demonstrate that *B. pseudomallei* enters the CNS via the olfactory and trigeminal nerves within 24 hr after inoculation. *Parameswaran, 2012, MJA, 196:345-348.

POS-TUE-200

THE COGNITIVE ASSESSMENT BATTERY (CAB) FOR HUNTINGTON'S DISEASE CLINICAL TRIALS

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Purpose: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease affecting cognition, voluntary movement, and psychiatric functions. Onset tends to occur in middle adulthood. Decades of research confirm that cognitive function declines in both the premanifest and early stages of (HD). Several clinical trials aimed at finding treatments for cognition in HD are in the planning stages and underway. As cognition has been prioritised as a primary outcome measure for treatments, cognitive markers of treatment effects and disease progression are essential. Here we report the development and reliability testing of a cognitive battery for clinical trials in HD. The aim of the Cognitive Assessment Battery (CAB) project was to capitalize on findings from previous studies to create and characterize a set of cognitive measures that can be applied in upcoming clinical trials for cognition in HD. Methods: The CAB study examined 250 participants (100 controls, 100 late premanifest, 50 early HD) from English-speaking sites in Australia, the US, Canada, and the UK, using a set of 14 cognitive tests that were repeated at each of three time points (2 consecutive days and six weeks later). Results: As expected, cognitive tests showed worse performance in CAG expanded subjects as compared to control subjects, with effect sizes (d) up to 2.13 in early HD and 0.80 in premanifest groups. Test-retest reliabilities across the battery ranged from 0.93 to 0.60. Practice effect profiles showed that the largest impact of practice occurred from the first to the second test exposure, although smaller declines in practice effects continued to be revealed in the third time point. Conclusions: The CAB battery provides researchers with a 60-minute cognitive battery that is scientifically justified, psychometrically well-characterised, and pragmatically feasible in the context of HD clinical trials.

POS-TUE-201

THE EFFECTS OF HYPOXIC PRECONDITIONING ON MYELINATION AFTER A NEONATAL HYPOXIC-ISCHAEMIC INJURY IN THE RAT

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Purpose: Myelination is an essential process in development that is carried out by oligodendrocytes in the central nervous system. Hypoxic-ischaemic (HI) events such as birth asphyxia can disrupt myelination by causing oxidative stress, inflammation, excitotoxicity, and disruption to normal mitochondrial function; resulting in the loss of myelin as well as oligodendrocytes. We have investigated the effects of hypoxic preconditioning on the process of myelination after a HI event. **Methods:** Sprague Dawley pups (postnatal day (P) 6) were placed in control and hypoxic preconditioned (8% oxygen, 3 hours) groups. On P7, pups were further separated into HI and sham surgery groups. HI surgery pups were anesthetised with 1.5% isofluorane and underwent a permanent unilateral right common carotid artery occlusion and then maintained at 8% oxygen for 3 hours. Sham pups underwent the same procedure without occlusion and were maintained in room air. Brains were removed 5 days post-surgery for histological analysis with cresyl violet and myelin basic protein (MBP) antibody. **Results:** HI alone (n=17) resulted in an increase in brain injury when compared to controls (n=8; 32.19±9.76% loss; $P<0.05$, 1-way ANOVA). Hypoxic preconditioning prior to HI (n=13) protected the brain from injury compared to HI alone (6.43±1.81% loss; $P<0.05$). HI alone also reduced the amount of myelin when compared to controls (40.11±8.81% loss MBP; $P<0.001$), while hypoxic preconditioning prior to HI prevented the loss of myelin compared to HI alone (14.75±2.84% loss MBP; $P<0.05$). **Conclusions:** These results indicate that hypoxic preconditioning not only reduces the degree of neuronal damage in the brain as a result of HI, but also protects against damage to myelin.

POS-TUE-202

PROTECTING THE GROWTH RESTRICTED PRETERM BRAIN FOLLOWING ANTENATAL GLUCOCORTICOIDS

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Purpose: Fetal intrauterine growth restriction (IUGR) is associated with increased neurological morbidity and mortality. IUGR infants are often born preterm and are therefore exposed to antenatal glucocorticoids to promote lung maturation. Melatonin acts as an antioxidant and may protect the fetal brain against oxidative damage. **Methods:** Pregnant ewes carrying twins underwent surgery at 0.7 gestation. In one fetus IUGR was induced via single umbilical artery ligation. Each twin was implanted with a carotid artery flow probe, electrocorticograph (ECoG) electrodes overlying the cerebral cortex and a femoral artery catheter. Betamethasone (BM; 11.4mg i.m. to ewe) or vehicle was given on days five (BM1) and six (BM2) following surgery. Melatonin administration (MLT; 2mg bolus, 2mg/hr i.v. to ewe) began with BM1. Post mortem was conducted on day seven; the fetal brain was fixed and processed for light microscopy. **Results:** At 14hrs post BM1 carotid blood flow was significantly increased in both IUGR+BM (49.5±15.9% increase, $p<0.001$) and IUGR+BM+MLT (62.2±21.9% increase, $p=0.009$) fetuses, compared to pre-BM. This timepoint corresponds to an increase in the amplitude of the ECoG signal intensity in IUGR+BM fetuses (13.1±6.5% increase) that does not occur in the IUGR+BM+MLT fetuses (2.2±5.5% increase). Within the fetal brain, the number of 4-HNE (lipid peroxidation) positive cells was increased in the cortex of IUGR+BM fetuses (25.9±11.2/mm^2) and reduced following MLT administration in IUGR+BM+MLT fetuses (4.9±3.7/mm^2). **Conclusion:** Melatonin does not prevent the rebound carotid blood flow reperfusion that occurs in IUGR fetuses exposed to antenatal betamethasone. However melatonin does prevent the increase in ECoG amplitude as well as oxidative stress within the fetal brain.

POS-TUE-203

INVESTIGATING NEURONAL SUBTYPES AND COLONIC MOTILITY IN THE NEUROLIGIN-3 R451C MOUSE MODEL OF AUTISM

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Gastrointestinal problems are reported in up to 90% of Autism spectrum disorder (ASD) patients. Multiple gene mutations affecting synaptic function are associated with ASD. Neuroligin-3R451C mice express a missense mutation in the nlgn3 gene coding for the neuroligin-3 postsynaptic adhesion protein and show altered GABA-mediated colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition between colonic migration motor complexes (CMMCs). Some NOS neurons also express GABA. Purpose: To determine whether NOS-mediated colonic motility is altered in NL3R451C mice and if changes in motility correspond to altered proportions of GABA and/or NOS neurons in NL3R451C mice colon. Methods: Colons were isolated from C57/Bl6, NL3R451C and WT (C57/Bl6-sv129-J) mice. Effects of the NOS inhibitor L-Nitro-arginine (NOLA, 100µm) on colonic motility were examined using video imaging techniques. Immunohistochemistry for Hu (a pan-neuronal marker), GABA and NOS was conducted on whole-mount myenteric plexus preparations from NL3R451C and WT colon. Results: Application of NOLA increased CMMC frequency in C57/Bl6 colons (n=8; $p < 0.05$). Similarly, NOLA increased CMMC frequency in NL3R451C colons (n=9 in each group; $p < 0.05$). In contrast, CMMC frequency was unaffected by NOLA in WT littermates (n=9). Furthermore, when compared to WTs, C57/Bl6 colons showed increased CMMC frequency in response to NOLA ($p<0.05$).The proportion of GABA or NOS immunoreactive neurons in WT (n=3) and NL3 R451C (n=3) mice was unchanged. Conclusion: These results suggest that the NL3R451C synaptic mutation alters nitric oxide-mediated colonic motility and that sensitivity to NOLA is strain-specific. Altered colonic motility in NL3R451C is not due to altered GABA/NOS neuronal numbers.

POS-TUE-204

GLUCOSE ENTRY INTO THE CITRIC ACID CYCLE IS DECREASED IN EPILEPTIC MICE

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Purpose: We characterized glucose and citric acid cycle (CAC) metabolism in a chronic epilepsy model as potential new treatment target for epilepsy. **Methods:** The pilocarpine mouse model was used. It results in spontaneous recurrent seizures in mice that experienced pilocarpine-induced status epilepticus (SE), but not in mice without SE. To assess the activity of glycolytic and CAC enzymes, crude mitochondrial extracts for enzyme activity assays were prepared from forebrain at the three day and three week post-SE time points, respectively. Also, three weeks after SE [1,2-13C]glucose (i.p) was administered before 5KW microwave irradiation of the head. Cortex and hippocampal metabolites were quantified through HPLC and CNMR. **Results:** At three days post-SE the activities of pyruvate dehydrogenase and pyruvate carboxylase were decreased by 23% and 28% respectively relative to no SE mice (n=9-12 mice per group, $p<0.05$). No statistically significant differences were found in the activities of aconitase and isocitrate dehydrogenase. Three week samples are currently being analysed. Cortical markers for pyruvate dehydrogenation pathway, namely the concentrations of [4,5-13C] glutamate (38%), [4,5-13C] glutamine(48%), [1,2-13C] aspartate (41%) and [3,4-13C] aspartate (41%), were reduced three weeks after SE (n=5-9, $p<0.01$-0.05). The concentrations of [2,3-13C] glutamate (50%) and [2,3-13C] glutamine(45%), markers for the pyruvate carboxylation pathway, were lowered (n=4-8, $p<0.001$-0.05). In addition, SE mice showed reduced levels of glutathione (18%), glutamate (21%) and alanine (32%) and increased levels of lysine (51%) and threonine (18%) in the hippocampal formation compared to mice without SE (n=10-11, $p<0.05$-0.001), consistent with reduced CAC activity. **Conclusion:** These results suggest that the pyruvate dehydrogenation and carboxylation pathways are deficient in chronically epileptic mice, resulting in suboptimal ATP synthesis and potentially contributing to seizure generation.

POS-TUE-205

MPTP-INDUCED NEUROINFLAMMATION IS REDUCED IN INTERFERON-α RECEPTOR-1 (IFNAR1) KNOCKOUT MICE

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Purpose: Pathological features of neuroinflammation including increased pro-inflammatory cytokines, astrogliosis and microglial activation have all been described in Parkinson's disease (PD). Key players in the neuroinflammatory cascade are the type-I interferons (IFNs), however their role in PD has not been explored. Previously, we identified that IFNAR1-/- neurons display reduced type-I IFN signalling and are protected against rotenone-induced toxicity *in vitro*. This study further investigated the type-I IFNs in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. **Methods and Results:** Wildtype and IFNAR1-/- mice were exposed to MPTP (4x10mg/kg, 2 hourly intervals) before brains were harvested at day-3, -7, -10 and -21 for RNA, protein and histological analysis. QPCR analysis confirmed elevated levels of IFNβ in wildtype mice at day-3 compared to sham control brains. Increased immunohistochemical expression of phosphorylated Stat-3, a downstream mediator of type-I IFNs, was identified in dopaminergic neurons and glial cells of the substantia nigra (SNpc). Pro-inflammatory cytokines, IL-1β and TNFα were upregulated at day-7 in wildtype brains (n=6, $p<0.001$). Significantly, the 2-fold increase (n=3, $p<0.01$) in IL-6 expression detected at day-3 in wildtype brains was not identified in the IFNAR1-/- brains (0.69±0.41, n=3) with the mice also displaying reduced GFAP staining in the SNpc and striatum. Increased Mac-1 staining suggestive of microglial activation was detected in the IFNAR1-/- brains and may reflect an early neuroprotective response. Conclusion: These results further support a role for the type-I IFNs in mediating the pro-inflammatory response in PD. We propose that targeting the type-I IFNs may be beneficial in reducing neuroinflammation and thus limiting the neuronal degeneration in PD.

POS-TUE-206

SHORT TERM NEUROPROTECTIVE ACTIONS OF HYPOXIC POSTCONDITIONING IN A NEONATAL RAT MODEL OF HYPOXIC-ISCHAEMIC BRAIN INJURY

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Purpose: Neonatal hypoxic ischaemic (HI) brain injury has been shown to cause a range of debilitating conditions and in some cases, may result in death. Exposure to mild hypoxia before injury can prevent subsequent injury, and this protection may involve the induction of hypoxia-responsive genes, anti-inflammatory and anti-apoptotic mechanisms. In this experiment, we have studied whether post-injury treatment (or postconditioning = PC) with mild hypoxia can reduce brain injury. Methods: Postnatal day 7 Sprague Dawley rat pups were exposed to HI treatment, which included a unilateral carotid artery occlusion with hypoxia (7% oxygen for 3 hours). Non-injured controls (CT) underwent identical surgery procedures without occlusion. Postconditioning started 24 hours after HI and consisted of daily exposure to 8% oxygen for 1 hour for 5 days following surgery. Normoxic controls (NC) were exposed to room air for the same duration. Brain injury was quantified using cresyl violet staining and the difference in volume between the ipsilateral and contralateral hemispheres was measured. Results: Control animals did not show any visible injury (CT+NC: -0.17±1.26 mm^3, n=8; CT+PC: -1.47±1.05mm^3, n=8). HI+NC pups had significant injury affecting the ipsilateral hemisphere (-15.67±4.37mm^3, n= 15, $p<0.05$, 1-way ANOVA), and the injury was reduced by PC (HI+PC: -5.15±2.6mm^3, n=16, $p < 0.05$ 1-way ANOVA). Conclusion: This study demonstrates the short term neuroprotective actions of hypoxic PC in the brain after HI injury and confirmed that PC alone does not cause brain damage. Further studies will elucidate the mechanisms involved in this novel neuroprotective phenomenon, and examine the effects of PC on neurons and glial cells.

POS-TUE-207

ADMINISTRATION OF A SUBSTANCE P, NK1 RECEPTOR ANTAGONIST REDUCES ONSET OF EXPERIMENTAL L-DOPA INDUCED DYSKINESIA

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Purpose: Dyskinesia (uncontrollable abnormal movements) is a common debilitating side effect of L-DOPA treatment, the gold standard for treatment of motor dysfunction in Parkinson's disease (PD). Identifying a treatment that prevents dyskinesia, without interfering with the benefits of L-DOPA treatment, is therefore of great importance. Dyskinesia is associated with alterations in basal ganglia functioning due to dopamine denervation and oscillating stimulation of dopamine receptors during L-DOPA treatment. Within the basal ganglia, the neuropeptide substance P (SP) is increased following L-DOPA treatment. Inhibition of SP may therefore represent a potential target for anti-dyskinetic treatment. Accordingly, this study determined whether L-DOPA combined with a substance P, NK1 receptor antagonist (N-acetyl-L-tryptophan 3,5-bis(trifluoro methyl)benzyl ester; NAT) reduced dyskinesia following PD. **Methods:** Adult male Sprague-Dawley rats (n=45) were anaesthetised and PD subsequently induced by two stereotaxic injections of 6-OHDA into the medial forebrain bundle. Four weeks following induction of PD, daily treatment was administered for 3 weeks with saline, L-DOPA (6mg/kg) or L-DOPA and NAT (0.5 and 5 mg/kg). Animals were assessed for dyskinesia (Abnormal Involuntary Movement scale), L-DOPA induced turning behaviour, motor function (rotarod) and immunohistological changes. **Results:** Development of dyskinesia was observed only when greater than 80% of dopaminergic neurons in the substantia nigra were lost and L-DOPA treatment was administered. NAT treatment combined with L-DOPA significantly reduced the onset of mild to moderate dyskinesia ($p=0.0141$) whilst not interfering with L-DOPA improved motor function. **Conclusion:** Antagonism of the substance P, NK1 receptor may represent a novel therapy for reducing the onset of L-DOPA induced dyskinesia associated with PD.

POS-TUE-208

INTERSTITIAL WHITE MATTER NEURONS EXPRESSING GABAERGIC MARKERS ADJACENT TO THE CINGULATE AND FRONTAL CORTICES

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Purpose: Increased density of GABAergic interenurons located in the white matter underneath the prefrontal cortex has been identified in postmortem brains in schizophrenia and supports an early developmental component to the disorder. Little is known about why there is increased density of these GABAergic interstitial white matter neurons (IWMNs) in schizoprhenia, nor where they originate from and their neurobiology. To anwer these questions, rodent models of increased IWMN density need to be developed. In this regard, IWMNs express many neurochemical markers for GABAergic interneurons such as glutamic acid decarboxylase (GAD), calcium binding proteins (eg. parvalbumin) and neuropeptides (eg somatostatin). The aim of this project was to develop a preparation that allows anatomical and functional characterization of IWMNs in the rodents. **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). Two PVeGFP mice and 2 wild type mice were anaesthetized (Ketamine, 100mg/kg) and decapitated. The brain was rapidly removed and fixed in 4% parafomaldehyde for ~ 6h before being prepared for immunohistochemical processing for a range of GABAergic interneuronal markers. **Results:** Neurons immunoreactive for Parvalbumin and/or GFP were observed in sparse numbers in the white matter underneath the frontal and cingulate cortices of the mouse brain. These neurons displayed an oval shaped cell body some of which had one or two processes generally aligned parallel to the grey matter/white matter border. Similar neurons immunoreactive for GAD and neuronal nitric oxide synthase were also observed in the white matter. **Conclusion:** These results show that IWMNs can be identified in the PVeGFP mouse and thus allows future neurobiological and neurophysiological studies on the function and fate of these neurons with respect to their increased density in schizophrenia.

POS-TUE-209

A NOVEL APPROACH TO IMPROVING CLINICAL TRANSLATION: MAGNETIC RESONANCE IMAGING OF MCA STROKE IN THE SHEEP

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Purpose: Clinical translation of stroke therapies from animal models to human patients has been extremely poor to date. One reason for this may be the choice of animal model. Small animal predominate the pre-clinical literature however, large animal models may more accurately predict efficacy in humans. Accordingly, we have recently developed a model of MCA occlusion in the sheep where reperfusion can be achieved. The aim of the present study was to use MRI to characterise the stroke lesion following MCA occlusion in the sheep. Methods: Merino sheep (n=18) were subject to either sham surgery or MCA occlusion achieved by either diathermy (permanent) or the application of an aneurysm clip (2h occlusion) under Isoflurane anaesthesia. Brain tissue oxygenation, intracranial pressure (ICP), blood pressure and blood gases were recorded for 24hrs after the induction of stroke. At 24hrs animals underwent magnetic resonance imaging (T1, T2, FLAIR, DWI, MRA) followed by perfusion with cold tris-saline. Brains were then removed for infarct volume assessment by tetrazolium chloride (TTC) staining and then processed for histological assessment. Results: On MRI, the large MCA stroke was associated with marked midline shift and tonsillar herniation, in addition to profound cerebral oedema. This was accompanied by a significant increase in ICP and decrease in brain tissue oxygenation across the 24hr monitoring period. Conclusion: The sheep model of MCA occlusion produces many features of clinical stroke including raised ICP and hallmark features on MRI. Such findings emphasise the value of this model in pre-clinical development of potential therapeutic agents for the treatment of stroke.

POS-TUE-210

DIFFERENTIAL BEHAVIOURAL EFFECTS OF CORTICOSTERONE OR CANNABINOID AGONIST TREATMENT IN MATERNALLY SEPARATED RATS

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Purpose: Epidemiological studies have suggested that schizophrenia is caused by an early disruption, such as environmental stress, which increases vulnerability to late factors, such as social stress or drug abuse, i.e. the 'two-hit' hypothesis. The aim of this project was to study the interaction between neonatal maternal deprivation stress and chronic treatment with either the stress hormone corticosterone (CORT) or the cannabinoid receptor agonist, CP55,940 (CP). **Methods:** Two cohorts of rats were used in this study (n=9-12/subgroup). Wistar rat pups were either maternally separated (MS) from their mothers for 3 hours every day from postnatal day 2-14 or left undisturbed. From 8 to 10 weeks of age animals from cohort one received CORT or vehicle in their drinking water while cohort two received daily CP or vehicle injections. Behavioural testing started at 12 weeks of age and included Y maze, novel object recognition, sucrose preference, plus maze and prepulse inhibition of startle (PPI) for both cohorts. **Results:** Spatial memory in the Y-maze was significantly disrupted in male MS animals treated with CORT but it was not affected in animals treated with CP. Sucrose preference was decreased in female MS animals treated with CORT while it was decreased in male MS animals treated with CP. The CORT cohort showed no anxiety-like behaviour in the plus maze task while time on the open arm was decreased in the CP cohort after MS and/or CP treatment in male animals and this was most pronounced in the 'two hit' group. Interestingly, MS induced a baseline PPI deficit in the second but not in the first cohort. **Conclusions:** The data shows that the combination of two environmental insults increases the risks of developing behavioural abnormalities in adulthood. However, it seems that different behavioural areas are targeted depending on the second stressor. While the combination of MS and CORT exposure induced a significant deficit in spatial memory, the combination of MS and CP targeted areas that are more relevant to emotional behaviour and deficits were seen in the sucrose preference test as well as in the plus maze. Furthermore, results were highly sex-specific with male animals being more vulnerable towards the two 'hits'. Overall, the data could shed new light on the mechanisms by which either stress and/or cannabis abuse are involved in the development of neuropsychiatric disorders.

POS-TUE-211

ENDOPLASMIC RETICULUM STRESS ALTERS TAU PATHOLOGY IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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Aim: Two of the most common causes of dementia, Alzheimer's disease and Frontotemporal lobar degeneration, are both characterized by pathological, neurofibrillary tangles consisting of the protein tau.The aim of this study was to investigate the role of endoplasmic reticulum (ER) stress in the development and progression of tau pathology in vivo. **Methods**: Transgenic mice that over express mutant, human tau within neurons of the cortex and hippocampus were crossed with a mouse strain that shows significant ER stress due to the knockout of the vital ER chaperone, Sil1. These mice (n>4 per group) then underwent biochemical, histological and behavioural analysis at various ages. **Results**: Double transgenic mice that overexpressed human tau and lacked the ER chaperone Sil1 displayed alterations in tau phosphorylation, solubility, and aggregation. **Conclusions**: These results demonstrate that ER stress can alter the development of tau pathology in vivo. Further investigations are required to determine the exact mechanism through which this occurs.

POS-TUE-212

THE L-NIO MODEL: A NOVEL METHOD FOR INDUCING FOCAL ISCHEMIA IN THE RAT

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Purpose: Previous studies have shown that the middle cerebral artery occlusion (MCAo) model is highly variable. We therefore established an alternative high-throughput model of focal ischemia to investigate inflammation and repair in subcortical lesions. Injection of L-N5-(1-Iminoethyl)ornithine hydrochloride (L-NIO), an endothelial nitric oxide synthase (eNOS) inhibitor, into the brain causes vasoconstriction and resultant ischemia. We hypothesised that L-NIO-induced focal ischemia would generate ongoing neuroinflammation and motor functional impairments characteristic of stroke. **Methods:** Under isoflurane anaesthesia, male Sprague Dawley rats (300-350g) had their right jugular vein ligated and L-NIO injected directly into the striatum (2µmol L-NIO in 5µl saline). Sham animals received saline injections. To assess inflammatory responses, animals were euthanised 3, 7 or 14 days following L-NIO or saline injections (n=3-5/group) and immunohistochemistry was performed on fixed tissue. Motor function was assessed at baseline, 1 and 4 weeks post-surgery (n=7-9/group). **Results:** GFAP and Iba1 immunoreactivity increased following L-NIO-induced focal ischemia and remained elevated 3-7 days post-insult compared to sham ($P<0.05$), similar to the MCAo inflammatory response. Fluoro-jade C was present within the lesion 3-7 days post-insult indicating ongoing cell death. In addition, L-NIO-induced focal ischemia resulted in impaired forelimb use 1 and 4 weeks post-insult compared to controls ($P<0.05$). **Conclusion:** We have characterised a novel method of inducing focal ischemia in rats using the eNOS inhibitor L-NIO. This model results in ongoing inflammation and impaired motor function up to 4 weeks post-insult. We propose the L-NIO model as an ideal model to assess anti-inflammatory approaches post-stroke.

POS-TUE-213

STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN MESIAL AND NON-LESIONAL TEMPORAL LOBE EPILEPSY

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Purpose: Mesial temporal sclerosis (MTS) is the most common pathology observed in patients with drug resistant temporal lobe epilepsy (TLE), which is associated with loss of hippocampal volume and signal changes on MRI. However, a significant number of TLE patients have no structural lesion identified on MRI, despite hypometabolism on fluorodeoxyglucose-PET ([^{18}F]-FDG-PET). It is uncertain whether these represent distinct groups of patients, or different ends of the spectrum of mesial temporal lobe epilepsy. The current study aimed to compare the patterns of FDG hypometabolism and $GABA_A$/central benzodiazepine receptor binding (with [^{18}F]-flumazenil (FMZ) PET) in patients with MTS and non-lesional (NL) TLE. **Methods:** FDG and [^{18}F]-FMZ PET were acquired in 12 MTS and 19 NL TLE patients with well-localised EEG seizure onsets. Hippocampal volumes, FDG uptake and [^{18}F]-FMZ binding were calculated using region of interest analysis, and the PET images compared using Statistical Parametric Mapping (SPM). **Results:** A strong negative correlation was observed between epilepsy duration and FDG uptake in NL patients (ipsilateral r^2=-0.63 p=0.01, contralateral r^2=-0.57 p=0.03), but not MTS patients (ipsilateral r^2=-0.09 p=0.80, contralateral r^2=0.65, p=0.04). Similarly, a trend to a negative correlation was observed between ipsilateral hippocampal volume and epilepsy duration in the NL patients (r^2=-0.47, p=0.07), but not the MTS patients (r^2=-0.03, p=0.95). SPM analysis revealed more widespread hypometabolism throughout the ipsilateral temporal lobe in the NL patients, compared to the MTS patients, showing hypometabolism in the anterior and mesial temporal lobe, although there was significantly greater hypometabolism in the anterior mesial temporal lobe of MTS patients than in NL patients. MTS patients showed reduced FMZ binding in the parahippocampal gyrus, whereas in NL patients this was most reduced in the superior temporal gyrus, with a greater reduction in the periventricular white matter in MTS patients than in NL patients. Interestingly, the NL patients showed an area of hypometabolism in the contralateral superior temporal gyrus when compared with the MTS group, which was not replicated on FMZ images. **Conclusion** These results suggest differing pathophysiological mechanisms underlie mesial TLE and NL TLE, with MTS patients displaying specific mesial temporal abnormalities, compared with NL patients who display both mesial and neocortical temporal abnormalities.

POS-TUE-214

MODIFIED PROPRIOSPINAL INNERVATION AFTER COMPLETE SPINAL TRANSECTION ALLOWS RECOVERY OF WEIGHT-BEARING LOCOMOTION

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Permanent loss of motor and sensory functions generally accompanies severe spinal cord injury in the adult. In contrast, the developing nervous system can show pronounced spontaneous functional recovery, referred to as the infant lesion effect. We have studied this phenomenon using the developing South American opossum, *Monodelphis domestica*. Previous work showed that following complete spinal transection during early development, weight-bearing locomotion recovered. At very early ages this was associated with demonstrable growth of axons across the injury site, but at later stages functional recovery was still possible in the complete absence of supraspinal re-innervation (Wheaton et al., PLoS ONE, 2011). Here we show that changes in propriospinal innervation occur in the neuronal networks in spinal cords of *Monodelphis* injured at different ages: one week (P7; n=6) or 4 weeks (P28; n=8). Complete mid-thoracic spinal transection was used to sever all descending projections from the brain to the lumbar locomotor circuits; the animals grew to maturity before behavioural testing and quantitative neuronal labelling were carried out to assess recovery and spinal remodelling. P7-injured *Monodelphis* recovered near-normal, highly coordinated locomotion (BBB 15.7±0.8; Regularity index (RI) 93±2.7%). Axonal tracing revealed that long descending supraspinal axons and propriospinal fibres had grown across the injury site. Consequently, animals were able to swim using hindlimbs suggesting that supraspinal control had been reestablished. P28-injured *Monodelphis* recovered weight-bearing use of their hindlimbs but with only limited coordination (BBB 12.3±0.2; RI 38±3.4%; p<0.01 vs control). Strikingly, this recovery occurred in the complete absence of any type of fibres growing across the injury site and did not extend to hindlimb movement while swimming, suggesting a lack of supraspinal control in their locomotion. Further quantitative labelling studies revealed remodelling of neuronal networks in the spinal segments caudal to the injury site of P28- and P7-injured animals. mRNA studies have identified changes in the levels of neurotransmitter receptor expression in these same segments. Together these results demonstrate that rewiring of spinal circuits may be involved in functional recovery, particularly in the absence of supraspinal innervation. Understanding this reorganization may provide novel therapeutic targets.

POS-TUE-215

KINASE CONTROL OF TDP-43 ACCUMULATION IN MOTOR NEURON DISEASE

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Purpose: Recently TAR-DNA binding protein 43 (TDP-43) was identified as a major protein constituent of cytosolic inclusions in spinal cord of patients with motor neuron disease. Our studies have focused on the cellular mechanisms controlling TDP-43 translocation from the nucleus to the cytosol and its subsequent accumulation and neurotoxicity. **Methods:** Neuronal and astrocyte cultures were treated with inducers of cell stress by mitochondrial or proteasome inhibition. Cells were also transfected with C-terminal TDP-43 or full length TDP-43 constructs containing familial mutations. After treatment with cell stress inducers (three or more separate experiments with multiple replicates), cells were examined for heterogeneous ribonucleoprotein K (hnRNP K), TDP-43, and active forms of protein kinases by immunofluorescence and western blot. **Results:** Multiple kinase inhibitors that target JNK, GSK3 or CDK2 were found to prevent abnormal TDP-43 accumulation, including both endogenous and transfected TDP-43. CDK2 inhibition was also able to reverse TDP-43 accumulation. Activated forms of JNK, CDK2 and GSK3 co-localized with accumulated TDP-43 in stressed cells. Neuronal stress also induced phosphorylation and accumulation of hnRNP K, which bound to TDP-43. This was reversed by inhibition of JNK, GSK3 or CDK2. Moreover, transfection of motor neuron hybrid cells with mutated TDP-43 (A315T or Q331K) resulted in abnormal hnRNP K expression and/or phosphorylation when compared to WT-TDP-43. **Conclusion:** JNK, CDK2 and GSK3 control abnormal accumulation of TDP-43. This accumulation is mediated by phosphorylation of the TDP-43 binding protein, hnRNP K. Abnormal hnRNP K processing has been associated with many disorders, including neurodegeneration. Understanding the mechanisms controlling TDP-43 accumulation and neurotoxicity can reveal novel targets for therapeutic intervention.

POS-TUE-216

THE THERAPEUTIC POTENTIAL OF NEUROEPITHELIAL CELLS AFTER RAT SPINAL CORD INJURY

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Mammalian spinal cord injury is a neurological condition that results in the loss of both sensory and motor function within the central nervous system (CNS) as damaged cells (neurons) within the spinal cord are unable to regenerate. Recent research suggests that, in the rat, early embryonic spinal cord cells known as Neuroepithelial cells (NE) may have the potential to stimulate damaged corticospinal tract (CST) axons and encourage growth in the adult spinal cord after injury. Objective: To examine the growth promoting potential of NE cells at two embryonic (E) time points, on CST axons after adult rat spinal cord injury. Methods: Fischer (F344) rat NE cells were dissected from E10.5 and E11.5 embryos, dissociated and implanted into an adult female F344 rat spinal cord hemi-section injury (n=8). Six weeks post injury rats were injected with Biotin Dextran, to label CST axons. Two weeks after tracing, rats were sacrificed and tissue immunohistochemistry performed for Avidin Peroxidase. Total axonal numbers were analysed including axonal/cellular interactions. Results: Implanted NE cells generate both scarring and neural cell pockets within the implant site after spinal cord injury. Increased CST sprouting was observed within E11.5 NE cell implants but not E10.5 NE cell implants, with a significant increase in CST axonal number observed in E11.5 implants vs. controls. Sprouting axonal varicosities were most associated with the presence of mature neurons, as opposed to the presence of both microglia/macrophages and blood vessels. Conclusions: In E11.5 NE cell implanted rats, regenerating CST axons grow significantly further into the implant. However while neuronal rich regions vigorously stimulate growth, areas of scar tissue appear to form barriers preventing the passage of axons. Thus reducing the degree of scar formation may increase axonal growth within NE cell implants after rat spinal cord injury.

POS-TUE-217

ROLE OF INNATE IMMUNE COMPLEMENT ACTIVATION IN NEURODEGENERATIVE DISEASE

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Purpose: There is increasing evidence that neuroinflammation drives the progression of neurodegenerative disease. This study explored the expression of the innate immune complement system, and role of the potent inflammatory activation fragment, C5a, in two mouse models of neurodegenerative disease. **Methods:** Transgenic C57BL/6J hSOD1^{G93A} mice were used as a model of motor neuron disease (MND), and transgenic CBB6 R6/1 mice were used as a model of Huntington's disease (HD). Lumbar spinal cord (MND) and striatal brain tissue (HD) were obtained from transgenic mice and their wild-type littermates at various ages during disease progression, and were examined for expression of C3, C5, and the C5a receptor, CD88, using qPCR and immuno-blotting (n=6/age), and immunohistochemistry (n=3/age). Circulating levels of C5a were also measured in both disease models using an ELISA. Finally, the function of C5a-CD88 signalling in MND was investigated by utilising CD88-deficient hSOD1^{G93A} mice (n=7-9) and hSOD1^{G93A} mice treated with the specific CD88 antagonist, PMX205 (60ug/ml drinking water, n=12). **Results:** Marked upregulation of complement factors and CD88 mRNA and protein occurred in the lumbar spinal cord (MND) and striatum (HD) of these mice, along with elevated plasma C5a levels, as disease progressed. CD88 was expressed predominantly by microglia/macrophages surrounding regions of neuronal cell death in both models. hSOD1^{G93A} mice treated with PMX205 showed significantly reduced muscle weakness, and increased survival compared to untreated mice. Similar results were found with CD88-deficient hSOD1^{G93A} mice compared with CD88-sufficient hSOD1^{G93A} mice. **Conclusion:** Our findings demonstrate that complement activation, C5a generation, C5a receptor upregulation, and ultimately CD88 signalling are key events in these neurodegenerative models. Reducing C5a-mediated neuroinflammation could be an important therapeutic strategy to treat a wide variety of neurodegenerative diseases.

POS-TUE-218

COPPER METABOLISM AND BEHAVIORAL TESTING IN ATP7B GENE KNOCK-OUT MICE

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Wilson Disease (WD) is a common metabolic disorder, whereby neurons and liver cells die due to toxicity from intracellular copper. The mechanism of copper sedimentary is still unclear. Here, we studied the copper metabolism and behavior in Atp7b gene knock-out mice. Behavioral testing including locomotor activity test, hang wire test, pole test, swimming test and the rotarod test, revealed no significant neurological symptoms were found in 10 months of age Atp7b knock-out (KO) mice. The biochemical changes and the histopathology of Atp7b-KO mice are coincident with that in the hepatic form of WD, and the Atp7b-KO mouse is a valid animal model of the hepatic form of WD. The decreased expressions of copper transport protein 1 and divalent metal transporter 1 are play a protective role in hepatic copper metabolism of Atp7b-KO mice, which could reduce the copper intake by the hepatic cells. Acknowledgments: The authors sincerely thank Professor Svetlana Lutsenko for her kindly providing Atp7b gene knock-out mice.

POS-TUE-219

THE EFFECT OF METALS ON THE STABILITY OF APOLIPOPROTEIN E

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Apolipoprotein E (APOE) genotype is a major genetic determinant for the risk of developing Alzheimer's disease (AD). There are three different alleles, with the ε4 allele being a risk factor for AD, while the ε2 allele is protective relative to the ε3 allele that is the most prevalent in the community. In plasma, total ApoE and ApoE4 levels have been found to be significantly lower in AD patients. **Purpose:** Given the known role of metals in the pathogenesis of AD, this study aims to determine whether metals such as zinc, copper and iron can affect the stability of the different ApoE isoforms. **Methods:** We utilised human plasma (healthy control and AD; n=5/group) provided by the Australian Imaging Biomarker Lifestyle (AIBL) study. 10 µl of water or different metal solutions (zinc chloride, copper chloride, ferric chloride) were added to 10 µl of plasma samples, then incubated at 37°C for 4 hours. In a separate study, we also added trypsin into the reaction to facilitate the degradation of ApoE. In both studies, western blot was used to determine whether there was any differential effect on full length ApoE and associated degradation products as a function of ApoE allele status or metal treatment in the two subject populations. **Results:** Data shows that both metals (zinc, copper and iron) and trypsin lead to an isoform-specific degradation of ApoE. **Conclusion:** Metals are involved in the stability of ApoE, and our ongoing analyses and expanded studies aim to determine whether this phenomenon contributes to the pathogenesis of AD.

POS-TUE-220

THE BLOOD-BRAIN BARRIER IS COMPROMISED IN RATS WITH CHRONIC HEART FAILURE

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Purpose: The blood-brain barrier (BBB) is of vital importance to normal brain function. It acts as a barrier to the entry of circulating substances, including many toxins, into the brain. Insults such as brain trauma and stroke changes BBB integrity but the effects of diseases such as heart failure on BBB function are unknown. Hence, we investigated the effect of heart failure on BBB integrity. **Methods:** We used a rat model of chronic heart failure (ligating the left anterior descending coronary artery for 8 weeks) and assessed BBB integrity by infusing Evan's Blue and fluorescently labelled dextran (10kD) via the carotid artery. **Results:** Here we show that the BBB of rats with chronic heart failure is compromised such that we see significantly (P<0.05) greater amounts of Evan's Blue (50.4±13.1 vs 6.8±1.5 pixels) and 10kD dextran extravasation in the brain parenchyma in heart failure versus sham rats respectively. Since cytokines such as CCL2 have been shown to reduce tight junction protein expression we assayed for plasma CCL2 levels in our sham and heart failure rats. We found significantly (P<0.05) higher plasma CCL2 levels in heart failure (14.7±2.1ng/ml) compared to sham (1.8±0.7ng/ml) rats. In *in vitro* cell culture studies, we found that the application of CCL2 (100ng/ml) to microvascular endothelial cells resulted in translocation of the tight-junction protein Claudin-5 from the endothelial cell membrane to the cell cytoplasm. **Conclusion:** We show that the BBB is compromised in heart failure rats possibly due to the direct effect of cytokines such as CCL2 on tight-junction proteins.

POS-TUE-221

GENE EXPRESSION PROFILING OF ROTENONE-MEDIATED CORTICAL NEURONAL DEATH: EVIDENCE FOR INHIBITION OF UBIQUITIN-PROTEASOME SYSTEM AND AUTOPHAGY-LYSOSOMAL PATHWAY, AND DYSFUNCTION OF MITOCHONDRIAL AND CAL

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Purpose: To examine the transcriptomic profile of rotenone-mediated neuronal programmed cell death to obtain a more in-depth understanding of the temporal recruitment of cellular signaling. Method: Cultured cortical neurons were treated with 10 nM rotenone for 8h, 15h and 24h, and RNA was harvested for Illumina Mouse Ref8 Ver.1.1 arrays. The absolute data was analyzed using GeneSpring GX 7.3. Genes with fold change ± 1.2-fold against controls in at least one of three time point conditions were annotated using DAVID V6.7 and PubMed search.. For each treatment, the assignment of the arrays was as follow: 8h (n=3), 15h (n=3), and 24h (n=3) and control (n=3) and statistic analysis was performed using One-way ANOVA approach ($P \leq 0.05$) Results: Global gene profiling analyses revealed a list of nine affected biological pathways. These include programmed cell death, mitochondria dysfunction, calcium signaling, chaperones/co-chaperones, unfolded protein response, ubiquitin-proteasome system, autophagy-lysosomal pathway, glutathione metabolism, and nuclear factor erythroid 2-related factor 2 (Nrf-2) pathway. Conclusions: In the early event (≤8h), rotenone induces mitochondrial dysfunction and subsequently disrupts calcium homeostasis, glutathione metabolism and triggers unfolded protein response. In the later event (≥8h), rotenone upregulated apoptogenic genes and Nrf-2 pathway while downregulated chaperones, ubiquitin-proteasome system, and autophagy-lysosomal pathway.

POS-TUE-222

AGE-RELATED LYSOSOMAL ALTERATIONS PERTURB INTRACELLULAR COBALAMIN TRAFFICKING

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Previous data indicates lysosomes become dysfunctional in ageing post-mitotic cells. As transit through lysosomes is essential for utilization of cobalamin (Cbl), we proposed that ageing processes (lipofuscin accumulation, altered lysosomal pH and protease activity) impair intracellular Cbl transport. **Objective**. To perturb lysosome function in vitro and assess the impact on intracellular [^{57}Co]Cbl transport. **Design**. Human HT1080 fibroblasts and SH-SY5Y neurons were treated with either chloroquine (to increase lysosomal pH), leupeptin (to inhibit lysosomal proteases) or lipofuscin (to induce lysosomal lipofuscin loading). Cells labelled with [^{57}Co]cyanoCbl were lysed and fractionated and [^{57}Co] was measured in lysosomal fractions by gamma-counting. **Results**. As a percentage of total cellular [^{57}Co]Cbl, fibroblast lysosomal [^{57}Co]Cbl levels increased from 6.0 + 0.1% to 23.0 + 0.8% after chloroquine treatment, and to 19.1 + 0.7% after leupeptin treatment. Lysosomal [^{57}Co]Cbl was ~ doubled to 11.8% of total cellular [^{57}Co]Cbl after treatment with lipofuscin, and it is noteworthy that this was under conditions where only ~10% of the cells were significantly loaded with lipofuscin as detected by flow cytometry. Similar results were obtained in experiments using SH-SY5Y neurons; e.g., lysosomal [^{57}Co]Cbl levels were increased 12-fold with chloroquine treatment. Taken together, these data suggest that Cbl may become trapped in lysosomes under pathophysiological conditions that impair lysosomal function in ageing and neurodegenerative diseases. This is predicted to increase cellular levels of toxic metabolites homocysteine and methylmalonic acid due to diminished supply of methyl-Cbl to cytosolic methionine synthase and of 5-deoxyadenosyl-Cbl to mitochondrial methylmalonyl-coenzyme A mutase. **Conclusions**: These studies provide evidence that age-related lysosomal dysfunction significantly inhibits Cbl transport from lysosomes.

POS-TUE-223

INDUCED PLURIPOTENT STEM CELLS AS TOOLS FOR DISEASE MODELLING AND DRUG DISCOVERY IN ALZHEIMER'S DISEASE

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The use of induced pluripotent stem cells (iPSCs), whereby a patient's somatic cells can be reprogrammed to a pluripotent state by the forced expression of a defined set of transcription factors, has the potential to enable in vitro disease modelling and be used for drug discovery programs. Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that leads to a decline in memory and cognition. Fibroblasts were taken from AD patients (or non-AD controls) and cultured under specific conditions to generate iPSCs which were then provided with growth factors to allow differentiation into neurons. While AD-iPSCs were morphologically indistinguishable from control-iPSCs, differentiated cells showed differing responses to cellular stresses. Since these cells are derived from individual patients, the use of iPSCs have provided novel insights into disease pathogenesis, providing information on an individual's variations in the disease process and their cellular response to drugs. Using this system we have identified a number of drugs that protect AD neurons against the damaging effects of oxidative stress.

POS-TUE-224

OREXIN RECEPTOR TYPE 1 BLOCKADE ATTENUATES THE SPONTANEOUS ACTIVITY OF LPGI NEURONS DURING NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL SYNDROME IN RATS

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Purpose: Orexin is involved in morphine-induced physical dependence and withdrawal. The Lateral Paragigantocellularis (LPGi) is a key brain region implicated in the expression of somatic signs of morphine withdrawal syndrome. Orexin A and orexin type 1 receptor, which in turn are involved in opiate dependence and withdrawal has been found in LPGi nucleus. The role of Orexin type 1 receptor in LPGi neurons on the expression of opiate dependence in these neurons has not been studied yet. In this study the effect of Orexin type 1 receptor blockade on neural activity of LPGi neurons during naloxone-precipitated morphine withdrawal syndrome was investigated. **Methods:** Male Wistar rats (in each group, n=6) weighing 250–300 g were made dependent on morphine by adding the drug to their drinking water. The effect of intra-LPGi administration of selective orexin type1 receptor antagonist (SB 334867, 0.2 µL, 100 µM) on naloxone (2 mg/kg/ml)-precipitated neural activity of LPGi neurons was studied by extracellular single unit recording. **Results:** Our results indicated that intra-LPGi administration of selective orexin type1 receptor antagonist (SB 334867, 0.2 µL, 100 µM) significantly decreases naloxone precipitated activity of LPGi neurons in morphine dependent rats ($p<0.0001$) but has no significant effect on basal activity of these neurons. **Conclusion:** It seems that orexin plays a role in increased neural activity of LPGi neurons through affecting orexin type 1 receptor during naloxone precipitated morphine withdrawal syndrome. **Key words:** orexin type 1 receptor, SB-334867, extracellular single unit recording, LPGi, rat.

POS-TUE-225

ALTERED RESTING STATE NETWORKS IN PARKINSON'S DISEASE: AN EXPLORATORY RESTING FMRI STUDY

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Purpose: Clinical manifestations of Parkinson's disease (PD) may arise from neurophysiological changes within specific brain circuits. These brain networks may be identified as resting state networks (RSNs) using resting fMRI. These RSN's are spontaneous random fluctuations in Blood Oxygen level Dependent activity with strong temporal coherence, measured using resting fMRI. Purpose of the study is to generate and identify different RSNs in PD and to explore its alterations as compared to healthy controls. **Methodology:** Study subjects were 10 PD patients (2 females) and 8 controls (2 females), matched for age (47.5 ± 7.3yrs V/s 43.8 ± 8.9yrs respectively). All the subjects underwent resting state fMRI (eyes closed) under 3T MRI. The fMRI data analysis was carried out using Probabilistic Independent Component Analysis (ICA) as implemented in Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC: ver-3.10), from FSL (www.fmrib.ox.ac.uk/fsl). Independent components were observed & RSN's were identified. Dual regression analysis was performed to compare RSN's between subjects and results were observed at $p<0.05$ (multiple comparison corrected) adjusted for age and sex. **Results:** MELODIC analysis generated 27 RSN's from the subject's data. Among RSN's the default mode network, primary motor, Primary visual, extra striate visual, fronto-parietal network and cerebellar networks were identified. On comparison significant ($p<0.05$ corrected for multiple comparison) lower activity was observed at extra striate visual and fronto-parietal networks in PD patients as compared to controls. **Discussion:** Study was able to generate and identify the presence of various RSN's from PD resting fMRI dataset. Study observations of significant lower resting state activity in PD at specific networks may indicate impaired functional integrity of these RSN's. However studies with larger sample size are required for validation and on validation these altered RSN's may have a potential to be used as a biomarker in understanding the patho-physiology and PD disease progression.

POS-TUE-226

THE EFFECT OF *NIGELLA SATIVA* L. SEEDS ON MOOD, ANXIETY AND COGNITION OF ADOLESCENT

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Purpose: The present study was designed to observe whether there is any effect of NS on mood, anxiety and cognition in healthy adolescent male volunteers. Methods: 48 adolescent male aged 14 to 17 years were recruited and were divided randomly into group A (n=22) and group B (n=26). Group A took 500mg NS capsule once daily for four weeks. Group B followed similar treatment procedure to group A but with placebo instead of NS. All the volunteers were assessed for cognition with Modified California Verbal Learning Test-II (CVLT-II), mood with Bond-lader scale and anxiety with State-Trait Anxiety Inventory (STAI) at the beginning and after four weeks of either NS or placebo intake. Results: After 4 weeks of NS intake, there was statistically significant variation of mood within group A but there was not statistically significant variation between group A and B. No significant variation was found in state anxiety within groups and between group A and B but in case of trait anxiety, significant variation was found within group A but not between group A and B. In case of CVLT II, there was significant variation within A in immediate short-term recall at trial 4 and 5 whereas this difference was found only in case of trial 5 between group A and B. Within group A, short term-free recall, long-term free recall and long-term cued recall had statistical difference whereas between group A and B long-term free recall and long-term cued recall had statistical difference. No parameters had significant variation within group B after placebo intake for 4 weeks. Conclusion: We propose extensive study with different fractions of NS in animals for finding the active ingredient(s) of NS giving such activities.

POS-TUE-227

LEAD-INDUCED PROTEIN CARBONYLATION IN MICE BRAIN AND BLOOD WAS REDUCED BY AQUEOUS LEAF EXTRACT OF *THUNBERGIA LAURIFOLIA* (LINN.)

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Purpose: The study aims to investigate the effects of aqueous leaf extract of Thai medicinal plant *Thunbergia laurifolia* Linn. (TL) on lead-induced protein carbonylation, a prominent biomarker of oxidative damage in brain tissue and blood. **Methods:** Fifty-four of 8-week-old male ICR mice were divided into 9 groups (n = 6) with various treatments: 1-sodium acetate 1 g/L in drinking water (control); 2-lead acetate (Pb) 1 g/L in drinking water; 3-TL 200 mg/kg bodyweight (BW); 4-Pb+TL 100 mg/kgBW; 5-Pb+TL 200 mg/kgBW; 6-Pb+TL 400 mg/kgBW; 7-Pb+TL 800 mg/kgBW; 8-Pb+vitamin E 100 mg/kgBW and 9-vegetable oil (the same volume as in gr-8). Aqueous extract of TL was administered daily via tube gavage, for 60 consecutive days. Protein carbonylation was determined in brain homogenates and blood by measuring the reactivity of carbonyl groups with 2,4-dinitrophenylhydrazine (2,4-DNPH), measured the absorbance at 360 nm with a molar absorption coefficient of 22.0 L $mmol^{-1}$ cm^{-1}. **Results:** Mice received co-treatment of Pb in drinking water at 1 g/L with aqueous TL leaf extract at 100, 200, 400 and 800 mg/kgBW for 8 weeks were significantly ($P<0.05$) attenuate adverse effects of lead in dose-dependent manners both in brain and blood samples on protein carbonylation (for brain; 1-0.11±0.03, 2-0.33±0.11, 3-0.03±0.02, 4-0.18±0.12, 5-0.09±0.07, 6-0.06±0.02, 7-0.04±0.02, 8-0.12±0.02, and 9-0.05±0.04 µmol 2,4-DNPH/mg protein, for blood; 1-11.33±1.93, 2-32.60±0.67, 3-3.28±1.81, 4-18.02±1.89, 5-10.16±1.56, 6-5.05±1.04, 7-3.92±1.92, 8-12.26±1.20 and 9-5.12±1.32 nmol 2,4-DNPH/mg protein). **Conclusion:** The result indicated that aqueous TL leaf extract exhibits potent anti-carbonylative activity against lead poisoning in mice brain and blood.

POS-TUE-228

PERTURBATION OF GABAERGIC TRANSMISSION DURING A POSTNATAL CRITICAL PERIOD ALTERS BEHAVIOR IN PATH INTEGRATION TASK

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Rodents navigate their surroundings using cues derived from the environment and self-movement. To assess the role of vestibular input in providing idiothetic cues for spatial navigation, brainstem slice preparations of postnatal rats were subjected to whole-cell patch-clamp studies targeting GABAergic neurons of the medial vestibular nucleus (VN). High frequency stimulation induced long-term depression of GABAA receptor-mediated evoked IPSCs, peaking at P3 - P5 and hardly inducible by P14. These results suggest a postnatal period whereby GABAergic synapses in the VN can be manipulated. To further study the role of GABAergic transmission in VN on developing acquisition of spatial recognition, we implanted above the VN of postnatal day 1 (P1) rats with a slice of Elvax loaded with GABAA receptor agonist (muscimol) or antagonist (bicuculline). These pups were allowed to recover and were tested for dead reckoning, which is a path integration task, at the adult stage. When compared with the sham controls, rats pretreated with muscimol at P1 showed no significant difference in light, dark, and new location probe tests. However, treatment with bicuculline at P1 significantly prolonged the training days, searching time, returning time, heading angle, as well as number of errors in both dark and new location probe tests. Taken together, our data indicate that GABAergic transmission in neonatal VN determines the acquisition of spatial navigation at the adult stage.

POS-TUE-229

ON THE ANTIDEPRESSANT-LIKE EFFECT OF 17BETA-ESTRADIOL: INVOLVEMENT OF DOPAMINERGIC, SEROTONERGIC, AND (OR) SIGMA-1 RECEPTOR SYSTEMS

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Purpose: 17beta-estradiol has been reported to possess antidepressant-like activity in animal models of depression, although the mechanism for its effect is not well understood. The present study is an effort in this direction to explore the mechanism of the antidepressant-like effect of 17beta-estradiol in a mouse model(s) of behavioral depression (despair behavior). Method: Despair behavior, expressed as helplessness to escape from a situation (immobility period), as in a forced swim test in which the animals (male laca mice) are forced to swim for a total of 6 min, was recorded. Results: 17beta-estradiol produced a U-shaped effect in decreasing the immobility period in mice(n=24). It had no effect on locomotor activity of the animal. The antidepressant-like effect was comparable to that of venlafaxine (16 mg/kg, i.p.) (n=24). 17beta-estradiol also exhibited a similar profile of antidepressant action in the tail suspension test (n=24). When co-administered with other antidepressant drugs, 17beta-estradiol (5 microg/kg, i.p.) potentiated the antiimmobility effect of subeffective doses of fluoxetine (5 mg/kg, i.p.), venlafaxine (2 mg/kg, i.p.), or bupropion (10 mg/kg, i.p.), but not of desipramine (5 mg/kg, i.p.) or tranylcypromine (2 mg/kg, i.p.), in the forced swim test (n=78). The reduction in the immobility period elicited by 17beta-estradiol (20 microg/kg, i.p.) was reversed by haloperidol (0.5 mg/kg, i.p.; a D(2) dopamine receptor antagonist), SCH 23390 (0.5 mg/kg, i.p.; a D(1) dopamine receptor antagonist), and sulpiride (5 mg/kg, i.p.; a specific dopamine D(2) receptor antagonist) (n=48). In mice pretreated with (+)-pentazocine (2.5 mg/kg, i.p.; a high-affinity sigma-1 receptor agonist), 17beta-estradiol (5 microg/kg, i.p.) produced a synergistic effect. In contrast, pretreatment with progesterone (10 mg/kg, s.c.; a sigma-1 receptor antagonist neurosteroid), rimcazole (5 mg/kg, i.p.; another sigma-1 receptor antagonist), or BD 1047 (1 mg/kg, i.p.; a novel sigma-1 receptor antagonist) reversed the anti-immobility effects of 17beta-estradiol (20 microg/kg, i.p.) (n=48). Similarly, in mice pretreated with a subthreshold dose of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, a 5-HT1A serotonin receptor agonist), 17beta-estradiol (5 microg/kg, i.p.) produced an antidepressant-like effect (n=24). Conclusion: These findings demonstrate that 17beta-estradiol exerted an antidepressant-like effect preferentially through the modulation of dopaminergic and serotonergic receptors. This action may also involve the participation of sigma-1 receptors.

POS-TUE-230

PREMATURE ACCUMULATION OF DNA DAMAGE IN THE BRAIN OF WNIN OBESE RATS AS A POSSIBLE CAUSE OF THEIR REDUCED LONGEVITY

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Purpose: Wistar of National Institute of Nutrition obese (WNIN/Ob) rat is a strain with hyperphagia, hyperinsulinemia and hyperleptinemia, developed at the NIN, Hyderabad, India, from an 80 year old Wistar rat (WNIN) stock colony. These rats have reduced longevity (an average lifespan of 15-18 months in contrast to 36 months in normal WNIN rats). In order to establish this rat as a model of ageing related studies, we have looked into the extent of DNA damage in brain which is a hallmark of senescence. **Methods:** DNA damage was assessed at the single cell level in cortex and hippocampus of WNIN/Ob rats (n=6) and control WNIN rats (n=6) at 5 months and 14 months of age by Comet Assay. For this, we had homogenised specific brain parts to form single cell suspension. The cells were mixed with low melting agarose at 37°C and immobilised on agarose-coated slides on ice. Then the samples were processed step-by-step for neutral and alkaline electrophoresis, followed by staining with fluorescent dye SYBR Green. Soon after this images of single cells were captured using epifluorescence microscope and analysed using CometScore™ Freeware image analysis software. **Results:** The extent of single-strand as well as double-strand DNA breaks in cells of cortex and hippocampus of 5 months old WNIN/Ob rats were comparable with those seen in the 14 months old normal WNIN rats. **Conclusion:** Onset of significant DNA damage in different brain regions of WNIN/Ob rats at younger age is a plausible cause of reduced longevity observed in these animals.

POS-TUE-231

REGULATORY ROLES OF MICRORNAS IN MICROGLIA-MEDIATED NEUROINFLAMMATION

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Introduction: Microglia, the resident macrophages of the central nervous system (CNS) respond to detrimental signals such as neuronal injury and infection by releasing proinflammatory cytokines and chemokines to attract other immune cells to the site of injury. However it has been widely demonstrated that the chronic activation of microglia leads to neuronal damage due to the excessive release of these molecules, implicating microglia in exaggerating the neuronal cell death in a number of neurodegenerative diseases. Thus, understanding the mechanism of microglia-mediated inflammation is crucial towards developing neurodegenerative disease therapies. Micro RNAs, a family of small, nonprotein-coding RNAs, have emerged as novel epigenetic factors regulating protein expression. Recent reports demonstrate that miRNAs have roles in modulating immune responses. Thus, the present study was initiated to identify microRNAs and their target mRNAs which can modulate microglial inflammatory response. Recently, miRNA 200b has been shown to target several proteins including c-JUN, the substrate of JNK MAPK (mitogen-activated protein kinase) which mediates the release of proinflammatory cytokines by activated microglia. **Results:** In this study miR-200b expression has been localized in microglia and found to be altered in activated BV2 microglia. Loss-of-function and gain of function studies confirmed c-Jun to be the target of miR-200b. Overexpression of miR-200b resulted in a decrease in JNK expression and activity thus dysregulating the MAPK-JNK pathway. Knockdown of miR-200b resulted in an increase in the pro-inflammatory cytokines and iNOS. Conversely, overexpression of miRNA-200b led to a decrease in the pro-inflammatory cytokine and iNOS expression. Finally treatment of neuronal cells, MN9D with conditioned medium obtained from microglial cells, resulted in increased inflammatory-mediated cell death upon knockdown of miR-200b. Overexpression of miRNA-200b also reduced the phagocytic ability in activated microglia. Upon activation, microglia undergo morphological transformation due to actin cytoskeleton reorganization. Overexpression of miRNA-200b resulted in reduction of F-actin microspike projections in activated microglia thereby affecting their morphology. **Conclusion:** Taken together, these results demonstrate the important role of miR-200b in modulating the MAPK pathway via cJun which in turn affects different aspects of the inflammatory process accompanying microglia activation including cytokine response, NO production, phagocytosis and neuronal cell death. Thus, miR-200b may prove to be a useful target for developing therapeutic strategies to control microglial mediated inflammation in neurodegenerative diseases.

POS-TUE-232

EXPRESSION OF PROSTATE APOPTOSIS RESPONSE-4 (PAR-4) IN HUMAN GLIOMA STEM CELLS AND REGULATION DURING DRUG- INDUCED APOPTOSIS

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Background: Cancer stem cells (CSC) are believed to contribute to chemoresistance in many solid tumors including gliomas, but the mechanisms involved are not clearly understood. The lacuna in this area is attributed to lack of experimental models. We have reported an established cancer stem cell line, HNGC-2, derived from a human glioma tumor. Par-4 is extensively studied in various cancers, however, little is known about its role in cancer stem cells. **Purpose:** The study aimed to examine the involvement of Par-4 in drug-induced cytotoxicity in CSC. **Methods:** We used a panel of drugs- Lomustine, Carmustine, UCN-01, Oxaliplatin, Temozolomide and Tamoxifen (TAM) for testing cytotoxicity in HNGC-2 cells. **Results:** Tamoxifen induced apoptosis in HNGC-2 cells ($p<0.01$) and upregulated the expression of Par-4. The effect was also observed in primary cultures derived from GBM tumors (n=3). In HNGC-2 cells, apoptosis was confirmed by Parp-cleavage, Annexin V and propidium iodide staining, break down of mitochondrial membrane potential and caspase-3 activity. Par-4 was predominantly localized in nucleus and exposure to TAM resulted in upregulation of Par-4 in both cytoplasm and nucleus in HNGC-2 cells but primary cultures (n=3) displayed staining in membrane and cytosol that was co-localized with actin. Knock down of Par-4 by siRNA inhibited TAM-induced cell death suggesting the involvement of Par-4 during apoptosis ($p<0.05$). **Conclusions:**Our findings suggests that up-regulation of Par-4 in glioma stem cells renders it sensitive to drug-induced apoptosis. The levels of Par-4 can be explored for screening potential anti-cancer agents in CSC.

POS-TUE-233

EFFECT OF ACUTE AND CHRONIC HYPOBARIC HYPOXIA EXPOSURE ON ADULT NEUROGENESIS

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Hypobaric hypoxia is a mild stress occurs at high altitude due to decrease in partial pressure of oxygen. It causes neurodegeneration which further leads to memory impairment. Hippocampus was found to be more prone to hypobaric hypoxia in comparison to other brain regions. Recent findings showed existence of neuronal stem cells in adult brain but their inability to integrate in functional network after stress. Present study was designed to study the effect of hypobaric hypoxia on cell proliferation and cell survival after exposure. Adult male Sprague dawley rats (n=24) were simulated to hypobaric hypoxia in an animal decompression chamber at an altitude of 25000 feet for 0, 1, 7 and 14 days. After exposure rats were decapitated and brain was fixed with 4% PFA. Markers for cell proliferation (BrdU, DCX, Ki67) and cell survival was studied with immunohistochemistry. Result of present study showed that acute exposure (1 day) to hypobaric hypoxia increases adult neurogenesis in hippocampus whereas chronic stress reduces the same as evident from level of different markers. Survival of neuronal stem cell in dentate gyrus of hippocampus was also reduced 4 weeks after exposure. Role of Pax6 was also studied and it was found that level of neurogenesis is regulated by Pax6 on chronic exposure to hypobaric hypoxia. From all observations it can be concluded that although the neurogenesis increased on acute exposure but survival of stem cells decreased after stress. Also Pax6 found to regulate adult neurogenesis in conjugation with CREB during hypobaric hypoxia exposure.

POS-TUE-234

CHRONIC SPINAL INFUSION OF LOPERAMIDE AND EXAMINATION OF TOLERANCE IN A RAT MODEL OF POSTSURGICAL PAIN

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Purpose: Plantar incision in rat generates spontaneous pain behavior. Morphine used to treat severe postsurgical pain produces tolerance after long-term administration. However, whether loperamide, a potent mu-opioid agonist, produce tolerance after continuous spinal administration is not known. **Methods:** Coinciding with the onset of spinal infusion of loperamide or morphine, rats were subjected to plantar incision. Chronic spinal infusion of drugs was achieved using intrathecal catheters connected to osmotic minipump. Pain-related behaviour was assessed by Hargreaves apparatus (thermal hyperalgesia) and von Frey filaments (mechanical allodynia). Morphine and loperamide (0.5, 1 and 2 µl/h) produced analgesia was observed until 7th day post-plantar incision in Sprague-Dawley rats (n=48). **Results:** Morphine and loperamide produced dose-dependent analgesia. Loperamide with its highest dose produced significant ($P<0.05$) analgesia till 7th day. However, the highest dose of morphine produced inhibition of thermal hyperalgesia till 5th day and mechanical allodynia till 3rd day post-plantar incision. **Conclusion:** Morphine and loperamide produced analgesia in postsurgical pain, may be mediated through different mechanism. Furthermore, thermal hyperalgesia and mechanical allodynia are regulated by two different mechanisms. Prolonged analgesia of loperamide could probably be due sustained blockade of calcium channels. Chronic morphine possibly produced tolerance in rats, leading to decreased withdrawal threshold with time.

POS-TUE-235

EFFECT OF BONE MARROW STROMAL CELLS TRANSPLANTATION ON SENSORIMOTOR RESPONSES TO NOXIOUS AND NON-NOXIOUS STIMULI IN COMPLETE SPINAL CORD TRANSECTION INJURY RATS

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Purpose: Spinal cord injury (SCI) leads to a devastating cascade of events including anatomical, physiological and neurochemical changes often leading to neuronal cell death. Loss of motor and altered sensory function; development of chronic or neuropathic pain may develop depending on injury location and severity. Both human and rodent bone marrow stromal cells (BMSC) have been studied and demonstrated behavioral efficacy (BBB score) in many rodent SCI. We report the effect of BMSCs transplantation on sensorimotor to different stimuli and autonomic function in the complete spinal cord transection (CT-SCI, T13) injury in rats. Methods: Adult male Wistar rats were divided into Sham, SCI+Vehicle and SCI+BMSC groups. In ketamine and xylazine (60 and 10 mg/kg, respectively) anesthetized rats, laminectomy followed by complete spinal cord transection (T11 vertebrae) was done. BBB score, tail flick latencies (TFL) to various temperatures; hot plate latency (HPL); threshold of tail flick (TTF); acetone test (AT) and bladder control were assessed during 8 weeks. Rat BMSCs were cultured and identify the presence of specific cell-surface antigens (CD44, CD90, CD45 and HLAII) using flowcytometry. PKH26 labelled BMSCs (~2.5 x 105 cells) were transplanted post-SCI day 9 at the site of injury and rats were sacrificed at wk8. Results: BMSCs were non-hematopoietic and in undifferentiated state. There was a significant recovery in sensorimotor parameters (post-SCI wk2-8 in BBB score; wk6-8 in TFL, HPL, TTF and AT) by BMSCs transplantation. Bladder recovery was significantly faster ($p<0.02$) and lesion volume was reduced in SCI+BMSC group. BMSCs were observed in spinal cord around the injury site and were positive for neuronal, astrocytes and oligodendrocytes markers (ß-III tubulin, GFAP and Olig4, respectively). Conclusion: Our results suggest the beneficial effects of BMSCs transplantation on sensorimotor and bladder control in CT-SCI rats. The results are supported by the reduction of lesion volume and differentiation of BMSCs in neuronal and glial cells.

POS-TUE-236

IMPAIRMENT IN NEURONAL DEVELOPMENT DUE TO DEFICIENCY OF ENDOSOMAL SNARES VTI1A AND VTI1B

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Purpose: The purpose of this research was to identify the function of two SNARE genes *i.e. vti1a* and *vti1b* which are involved in early endosomal/ trans-Golgi network and late endosomal trafficking events respectively. **Methods:** Double knockout mice (DKO) was used for both *vti1a* $^{(-/-)}$ and *vti1b* $^{(-/-)}$ which was compared with double heterozygotes (DHET,$^{+/-,+/-}$). Routine histological techniques and immunohistochemistry was performed. Neurotracing dye DiI was used to label the thalamocortical and corticofugal fibers. In vitro studies were also carried out to investigate the neurite outgrowth pattern. **Results:** Numerous changes in central as well as peripheral nervous system (n=3) was noted in DKO mice. In CNS, they show wide ventricles and lacked several fiber tracts including anterior commisure, spinotrigeminal, corticospinal tracts, hippocampal as well as nigrostriatal fibers. Corpus callosum thickness was reduced and thalamocortical axons did not cross pallio-subpallial border. While, only a few corticothalamic fibres reached thalamus. Progressive neurodegeneration was observed in majority of DKO peripheral ganglia. Neurons were reduced by more than 95% in DKO dorsal root ganglia at embryonic day 18.5. **Conclusion:** Inability of endosomal membrane traffic in *vti1a* $^{(-/-)}$, *vti1b* $^{(-/-)}$ neurons was suggested by overall phenotype leading to absence or reduction in axonal fiber formation. *vti1a* $^{(-/-)}$ or *vti1b* $^{(-/-)}$ single deficient mice were viable without these neuronal defects, indicating that they can substitute for each other in these processes.

POS-TUE-237

CHARACTERISTICS OF PRRT2 MUTATIONS IN SPORADIC PAROXYSMAL KINESIGENIC DYSKINESIA: HETEROGENEITY, INCOMPLETE PENETRANCE AND DE NOVO MUTAGENESIS

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Background: Paroxysmal kinesigenic dyskinesia (PKD) is an episodic movement disorder characterized by choreoathetosis or dystonia which were usually triggered by sudden movement. Proline rich transmembrane protein 2 (PRRT2) was recently identified as a causative gene for PKD and c.649dupC mutation within PRRT2 was shown to be a frequent mutation in familial cases as well as in sporadic cases. The high frequency of c.649dupC mutation identified in sporadic cases might be attributed to the incomplete penetrance of this mutation or origin of de novo. The aim of this study is to summarize some features of PRRT2 mutations in sporadic PKD. Methods: Nine sporadic Chinese PKD patients including one Mongolian case were recruited. Direct sequencing of PRRT2 was performed in them and their parents. Haplotype analysis was conducted to confirm the biological relationship. Several constructs encoding GFP-tagged wild-type PRRT2 (WT PRRT2-GFP), missense PRRT2 (c.796C>T, c.841T>C, c.859G>A et al.) and truncated PRRT2 (c.649dupC, c.514-517delTCTG, c. 964delC et al.) were generated respectively in CHO and SH-SY5Y cell lines. Results: A novel mutation, c.133_136delCCAG, was identified in one Han patient and his unaffected mother. The c.649dupC mutation was detected in another two Han patients and their respective unaffected father. To our interest, c.649dupC was also detected in the Mongolian patient but not in his parents. Haplotype analysis confirmed the biological relationship among the trio. No mutations were identified in the remaining 6 patients. Truncated PRRT2-GFP lost membrane targeting and was located in the cytoplasm, while WT PRRT2-GFP and missense PRRT2-GFP were localized in membrane. Conclusions: Heterogeneity, incomplete penetrance and de novo mutagenesis were identified as characteristics of PRRT2 mutations in sporadic PKD. Our results also indicated that truncated PRRT2 mutations were clearly pathogenetic, while missense PRRT2 mutations might be not. Keywords: paroxysmal kinesigenic dyskinesia; PRRT2; de novo; mutagenesis.

POS-TUE-238

NEUREGULIN 1 REGULATES EXCITABILITY OF FAST-SPIKING NEURONS THROUGH KV1.1 AND ACTS IN EPILEPSY

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Purpose : Epilepsy is a disabling neurological disorder that affects about 1% of the general population of all ages. About 30% of affected individuals continue to have breakthrough seizures despite appropriate pharmacological anticonvulsant treatment, and surgical removal of the epileptic focus is suitable only for a minority.Dysfunction of fast-spiking, parvalbumin-positive (FS-PV) interneurons is implicated in the pathogenesis of epilepsy. ErbB4, a key Neuregulin 1 (NRG1) receptor, is mainly expressed in this type of interneurons, and recent studies suggest that parvalbumin interneurons are a major target of NRG1-ErbB4 signaling in adult brain. Thus, we hypothesized that downregulation of NRG1-ErbB4 signaling in FS-PV interneurons is involved in epilepsy. **Methods**: We combined electrophysiological and pharmacological approach to determine whether stimulation or disruption of NRG1-ErbB4 signaling affect the excitability of PV cells. We also use convulsant agent to induce mouse models to evaluate the seizure susceptibility with Pvalb-cre;Erbb4 $^{loxp/loxp}$ mice and Erbb4 $^{loxp/loxp}$ mice. **Results**: We found that NRG1, through its receptor ErbB4, increased the intrinsic excitability of FS-PV interneurons. This effect was mediated by increasing the near-threshold responsiveness and decreasing the voltage threshold for action potentials through Kv1.1, a voltage-gated potassium channel. Furthermore, mice with specific deletion of ErbB4 in parvalbumin interneurons were more susceptible to pentylenetetrazole- and pilocarpine-induced models of epilepsy. Exogenous NRG1 delayed the onset of seizures and decreased their incidence and stage. **Conclusion**: Our findings suggest that ErbB4 may be a target for the development of a new class of anticonvulsant drugs.

POS-TUE-239

MEC-17 DEFICIENCY LEADS TO REDUCED α-TUBULIN ACETYLATION AND IMPAIRED MIGRATION OF CORTICAL NEURONS

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Neuronal migration is a fundamental process during the development of the cerebral cortex and is regulated by cytoskeletal components. Microtubule dynamics can be modulated by post-translational modifications to tubulin subunits. Acetylation of α-tubulin at lysine 40 is important in regulating microtubule properties, and this process is controlled by acetyltransferase and deacetylase. MEC-17 is a newly discovered α-tubulin acetyltransferase that has been found to play a major role in the acetylation of α-tubulin in different species in vivo. However, the physiological function of MEC-17 during neural development is largely unknown. Here, we report that MEC-17 is critical for the migration of cortical neurons in the rat. MEC-17 was strongly expressed in the cerebral cortex during development. MEC-17 deficiency caused migratory defects in the cortical projection neurons and interneurons and perturbed the transition of projection neurons from the multipolar stage to the unipolar/bipolar stage in the intermediate zone of the cortex. Furthermore, knockdown of α-tubulin deacetylase HDAC6 or overexpression of tubulin^{K40Q} to mimic acetylated α-tubulin could reduce the migratory and morphological defects caused by MEC-17 deficiency in cortical projection neurons. Thus, MEC-17, which regulates the acetylation of α-tubulin, appears to control the migration and morphological transition of cortical neurons. This finding reveals the importance of MEC-17 and α-tubulin acetylation in cortical development.

POS-TUE-240

A NOVEL SYNTHETIC CURCUMINOID DERIVATIVE ATTENUATES NEUROPATHIC PAIN IN MICE VIA NO-INDEPENDENT cGMP ACTIVATION OF K_{ATP}

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The clinical condition of neuropathic pain has been dramatically described as “the most terrible of all tortures, which a nerve wound may inflict”. Recently, our research group has fabricated a novel synthetic curcuminoid analogue, namely 2,6-bis-(4-hydroxy-3-methoxybenzylidene)-cyclohexanone or BHMC which was reported effective in inhibiting acute models of neurogenic and inflammatory pain in mice. The present study investigated the role of nitric oxide (NO) cyclic guanosine monophosphate (cGMP) and ATP sensitive potassium channel (K_{ATP}) pathway in anti-hyperallgesic effect of BHMC in chronic constriction injury (CCI) model of neuropathic pain. Briefly, CCI mice were produced via surgical ligation of left sciatic nerve of each mouse under anaesthesia. After 14 days, the anti-hyperalgesic effect of BHMC in CCI model was evaluated using mechanical Randall Sellito test. CCI-induced mice (n=8) were pre-treated with non-selective nitric oxide synthase inhibitor, L-NAME (10 mg/kg, i.p.), selective nitric oxide-sensitive guanylyl cyclase inhibitor, ODQ (2 mg/kg, i.p.) or selective ATP-sensitive K^+ channel blocker, glibenclamide (10 mg/kg, i.p.) before administration of BHMC (1 mg/kg, i.p.). It was demonstrated that pre-treatment with ODQ and glibenclamide, but not L-NAME significantly reversed inhibitory effect of BHMC on CCI-induced neuropathic pain. Thus, based on these results, it was suggested that BHMC-induced analgesia in CCI mice is mediated via NO independent activation of cGMP-induced K_{ATP} opening, leading to hyperpolarisation in nociceptive neurons. Further dissection of the mechanism of analgesic action of BHMC are required to establish BHMC as prospective analgesic agent clinically.

POS-TUE-241

MONTELUKAST MODULATES THE PROTECTIVE EFFECT OF ROFECOXIB AND CAFFEIC ACID AGAINST KAINIC ACID-INDUCED COGNITIVE DYSFUNCTION IN RATS

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Purpose: Mild cognitive impairment (MCI) serves as a prodrome to Alzheimer's disease. Antioxidants and COX-2 (cyclo-oxygenase-2) inhibitors have also been reported to have beneficial effects against conditions of memory impairment. Thus, the present study purports to explore the potential role of montelukast (a cysteinyl leukotriene inhibitor) in concert with rofecoxib (COX-2 inhibitor) and caffeic acid (a 5-LOX inhibitor and potent antioxidant) against kainic acid induced cognitive dysfunction in rats. **Methods:** In the experimental protocol, kainic acid (0.4µg/2µl) in Artifiscial Cerebrospinal Fluid (ACSF) was given intrahippocampally (CA3 region) to induce a condition similar to MCI. Memory performance was measured on the day 10-14 and the locomotor activity was measured on the day 1, 7 and 14. For estimation of biochemical, mitochondrial and histopathological parameters, animals were sacrificed on day 14, stored at -80 degree centigrade and the estimation was done on the 15th day. **Results:** The treatment groups consisting of montelukast (0.5 and 1 mg/kg), rofecoxib (5 and 10 mg/kg) and caffeic acid (5 and 10 mg/kg) showed significant improvement in memory performance, oxidative stress parameters and mitochondrial function as compared to that of control (kainic acid treated), however, combination of montelukast with rofecoxib showed significant improvement in their protective effect as compared to the Montelukast and caffeic acid group. **Conclusion:** The present study emphasizes the positive modulation of cysteinyl leukotriene receptor inhibition on COX (cyclooxygenase) and LOX (lipoxygenase) pathways in the control of the neuroinflammation in kainic acid induced cognitive dysfunction in rats.

POS-TUE-242

NEUROGENIC DIFFERENTIATION OF RAT FULL TERM AMNIOTIC FLUID STEM CELLS (AFSCS)

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Purpose: Stem cells technology has been by far the most exciting discovery of the era which aims for cell therapy for various diseases ranging from genetically linked disorders to neurodegenerative diseases as well as injuries. However, from research perspective, there is no best stem cells candidate available at this moment as each has its own limitations and advantages. Therefore, finding the right stem cells in generating the best differentiated cell type for therapeutic application is indeed essential. Recent finding discovers that amniotic fluid serves as an excellent alternative source of pluripotent stem cells, as they are not bound with ethical issues and are more primitive than adult stem cells, hence their potential is higher. We have managed to isolate, the best of our knowledge the first, high potency stem cells from rat full term amniotic fluid. The cells have been shown to express c-Kit (a marker for stem cells receptor), Oct4 (pluripotency marker) and tert (telomerase reverse transcriptase for unlimited proliferation), and more importantly have the ability to form multicellualr aggregates, embryoid bodies (EBs), suggesting the wider differentiation spectrum of the cells including neural lineages. In this experiment, we aim to discover the neurogenic potential of rat full term amniotic fluid stem cells (AFSCs) upon prolonged culture in monolayer differentiation. **Method:** AFSCs were subjected to monolayer differentiation (Ying *et al*., 2003). Medium was changed every 2days for two weeks. On days 2, 4, 6, 8, 10, 15, cells were harvested for RT-PCR and immunocytochemistry (ICC) with respective markers, namely early neuronal markers (PAX 6 and Nestin), post-mitotic neuronal markers (beta tubulin III) and mature neuronal markers (Neurofilament, MAP2, GFAP and TH). **Results:** Upon 2-hours of induction, rat full term AFSCs showed early neuronal marker (nestin and PAX-6). After 2days, beta tubulin III was observed. GFAP expression was observed from day 4 to day 10. Neurofilament, MAP2 and TH were observed started from day-10 onwards. **Conclusion:** Rat full term AFSCs harbor strong neurogenic capacity and upon simple induction protocol, namely monolayer differentiation, AFSCs could be potentially become a tool in understanding the basic mechanisms involved in neural differentiation process *in vitro*.

POS-TUE-243

MICROGLIAL SIRTUIN 3 REGULATES REACTIVE OXYGEN SPECIES PRODUCTION IN NEUROPATHOLOGY

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Purpose: Microglia, being the immune cells of the central nervous system (CNS) respond to injuries, infections and other degenerative stimuli in CNS. Chronically activated microglia are known to secrete excessive amounts of reactive oxygen species (ROS), causing oxidative stress thereby resulting in neurodegeneration. Sirtuin 3 (Sirt3), a member of the protein deacetylating enzymes, mediates the antioxidant defense mechanism and protects tissues from oxidative damage. In this context, we investigated the expression and function of Sirt3 in microglia activated by inflammation, brain injury and Alzheimer's disease (AD). **Methods and Results:** Sirt3 expression was found to be increased following activation of microglia with lipopolysaccharide (an endotoxin) both *in vivo* and *in vitro* . Knockdown of Sirt3 in microglia resulted in an increased cellular and mitochondrial ROS and decreased the expression of superoxide dismutase (Sod), an antioxidant gene suggesting that Sirt3 regulates the ROS production by elevating the expression of antioxidants. Cellular Sirt3 has been shown to be into exist in two forms (short and long). Overexpression of these two different Sirt3 protein forms separately in microglia increased the expression of the antioxidant gene catalase by upregulating the expression of a transcription factor, Foxo3a. Chromatin immunoprecipitation studies revealed that FOXO3a binds to the promoters of catalase and Sod in microglia, suggesting that Sirt3 regulates ROS in microglia *via* FOXO3a-mediated antioxidant defense mechanism. All experiments involved at least 3 samples (n=3). In the in vivo traumatic brain injury rat model, Sirt3 expression was markedly induced in the activated microglia on the injured side of the brain as opposed to its hardly detectable expression in the ramified microglia on the non-injured side. Contrary to this, the expression of Sirt3 was increased only in the neurons but not in the microglia of AD-induced rat brains. **Conclusion:** These results suggest that Sirt3 is induced in microglia after acute injury but not in chronic neurodegenerative conditions, indicating lack of microglia-mediated neuroprotection against oxidative damage in neurodegenerative diseases.

POS-TUE-244

INFLUENCE OF FORCED SWIM STRESS ON NEUROBEHAVIORAL TOXICITY OF LAMBDA-CYHALOTHRIN IN RATS

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Purpose: Human may encounter with environmental neurotoxicant and stress both in occupational & non occupational settings. The present study has been carried out on rats to assess the role of forced swim stress (FSS) on the neurobehavioral toxicity of lambda-cyhalothrin (LCT), a new generation type II synthetic pyrethroid with extensive applications. **Methods:** Rats were subjected to FSS (15 min/day) or exposed to LCT (0.5 mg/kg body weight, p.o.) or simultaneously exposed to FSS and LCT for 28 days. Effect on grip strength, learning and memory was assessed by grip strength meter and Y maze. Plasma corticosterone was estimated by RP-HPLC. Activity of acetylcholine esterase, sensitivity of cholinergic-muscarinic receptor and expression of choline acetyltransferase (ChAT) was assessed in hippocampus and frontal cortex following standard protocols. **Results:** Rats subjected to FSS exhibited decrease in learning and memory compared to controls. Increased levels of plasma corticosterone associated with decreased the binding of cholinergic-muscarinic receptors and protein expression of ChAT in hippocampus and frontal cortex was observed in rats subjected to FSS, compared to controls. Marginal changes in these behavioural and neurochemical parameters were observed in rats treated with LCT compared to controls. Simultaneous exposure to FSS and LCT caused marked change in plasma corticosterone levels, behavioural and neurochemical parameters as compared to rats exposed to FSS, LCT or those in the control group. **Conclusion:** The results exhibit that physical stressor (FSS) could be a contributing factor in enhanced neurobehavioral toxicity of lambda-cyhalothrin.

POS-TUE-245

ALTERED LEVELS OF NEUROTROPHIC FACTORS AND NEUROCHEMICAL PROFILE IN THE BRAIN AS THE PROBABLE CAUSES OF DECREASED LONGEVITY OF WNIN OBESE RATS

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Purpose: Wistar NIN obese (WNIN/Ob) rats developed at the National Institute of Nutrition are the heaviest inbred rat strain in the world. These rats are hyperphagic, hyperinsulinemic, hyperleptinemic and have reduced longevity (an average lifespan of 15-18 months in contrast to 36 months in normal Wistar rats). In the WNIN/Ob rats, we intend to delineate the factors responsible for reduced longevity. **Methods:** Neurotrophic factors are responsible for the survival of developing neurons and the maintenance of mature neurons. We have estimated levels of key neurotrophic factors using BioPlex assay and done neuro-glial profiling using Immunohistochemistry in different brain regions of these rats (n=6). As Glutamate (Glu) and Gamma-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in mammalian CNS, we have looked at the levels of these neurometabolites in different brain regions of WNIN/Ob rats (n=4) and their age matched normal rats (n=4) using Magnetic Resonance Spectroscopy (MRS). We have evaluated if there are any volumetric differences in the brain of WNIN/Ob rats in contrast to the age matched controls using Magnetic Resonance Imaging (MRI). **Results:** Our findings show that the levels of key neurotrophic factors like BDNF and IGF-1 are altered in the WNIN/Ob rats. MRS data indicates hypo-metabolism in the brain of WNIN/Ob rats. But there are no significant volumetric changes in the brain of the WNIN/Ob rats when compared to controls. **Conclusion:** Altered levels of neurotrophic factors and neurochemical profile in the brain are one of the many factors behind decreased longevity of WNIN obese rats.

POS-TUE-246

REGULATION OF GLUTAMATE MEDIATED EXCITOTOXICITY IN TEMPORAL LOBE EPILEPTIC RATS THROUGH AKT ACTIVATION: ROLE OF WITHANIA SOMNIFERA AND WITHANOLIDE A

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Purpose: To study the role of Withania somnifera and Withanolide A in regulating glutamate mediated excitotoxicity in pilocarpine model of Temporal lobe epileptic rats. Methods: Cellular degeneration in hippocampal sections was studied using Nissl staining. Behavioural assessment was performed using Y maze test. Glutamate synthesis, metabolism and transport was studied using mRNA expression of GLAST and GAD using Real Time PCR and Spectrophotometric assay of GDH activity. AMPA receptor function was analysed using receptor binding study, mRNA expression and immunohitochemical analysis. The cell survival enhancing property of WS was studied using expression of BAX, Caspase 8, and Akt along with immunohitochemical analysis of Phospho Akt. Results: The WS and WA treated epileptic rats had enhanced Nissl Staining indicating more number of revived neurons in the hippocampus. This result was in accordance with the Y Maze test, in the form of regained performance which was observed in WS and WA treated epileptic rats. Glutamate metabolism and transport was disturbed in epileptic rats, which is evident from altered GDH activity, Gad expression and GLAST expression, leading to excessive accumulation of glutamate. AMPA receptor binding studies and Immunohistochemical analysis revealed altered receptor function. WS and WA restored the altered GDH activity, Gad expression, GLAST expression & AMPA receptor density to near control. An enhanced secondary messenger IP3 level was also observed in epileptic rats, which could have escalated cytosolic calcium levels. Neuronal death through apoptotic mechanism was noticed through increased expression of BAX and Caspase 8 in epileptic rats. In WS and WA treated rats enhanced expression of Akt was observed, suggesting activation of anti apoptotic mechanisms resulting in downregulation of BAX and Caspase 8 expression attenuating neuronal loss. Conclusion: Our results propose neuromodulatory effect of WS & WA in pilocarpine model of TLE.

POS-TUE-247

EFFECT OF DEXAMETHASONE ON OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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Dexamethasone (Dex) is a synthetic glucocorticoid that widely used for several therapeutic in humans. The side-effects of Dex exposure leads to changes in brain function. However, the effects of Dex on neurons are still not completely understood. **Purpose:** Investigate the effect of Dex on neuronal calcium levels, mitochondrial membrane potential, oxidative stress and mitochondrial dynamics. **Methods:** Evaluated the effect of Dex on intracellular calcium concentration by using fura2 AM. **Results:** We found that oxidative stress induced by Dex resulting in increase in lipid peroxidation and reduction in the activity of glutathione peroxidase. 1 μM Dex decreases intracellular calcium levels that coincided with mitochondrial membrane potential depolarization. The effect on mitochondrial fission-fusion imbalance in Dex caused abnormal mitochondrial functions. Dex exposure increased the percentage of neuronal death in dose and time-dependent manner. **Conclusion:** These results demonstrate that Dex-induced neuronal death through a mechanism that involves increases in oxidative stress and altered balance in mitochondrial fission and fusion.

POS-TUE-248

ANTIGENOTOXIC ACTIVITY AND NEUROPROTECTIVE POTENTIAL OF *CENTELLA ASIATICA* (L.) URBAN

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Background & Purpose: Oxidative stress has been etiologically implicated in several human diseases specially cancer, Alzheimer's disease, Parkinson's disease, and also in aging. Extracts/pure compounds isolated from natural medicinal plants have been tested for their antioxidant and neuroprotective properties in many studies. *Centella asiatica* (L.) Urban, is a reputed medicinal plant mentioned in Indian literature that possess various pharmacological effects favorable for human health. The present study has been planned to envisage the antigenotoxic activity and neuroprotective potential of various extracts from *Centella asiatica* (L.) Urban. **Methods:** In the present study the whole plants of *Centella asiatica* (L.) Urban were used and extracted/fractionated for Methanol Extract (CME), Hexane Fraction (CHF), Chloroform Fraction (CCF), Ethyl acetate Fraction (CEA). Antigenotoxic activity of these extract was tested against hydrogen peroxide induced DNA damage using SOS chromotest. Further, the neuroprotective potential of most active component i.e. methanol extract was evaluated using MTT assay and glial fibrillary acidic protein (GFAP) expression in differentiated C6 cells. **Results:** Methanol extract (CME) significantly decreased the SOSIP of H_2O_2 by 83%. The potency of various isolates as assessed from their percent of inhibition of genotoxicity in decreasing order is CME > CHF > CCF > CEA. Promising results were seen the neuroprotective potential of CME in MTT assay and GFAP expression in differentiated C6 cells. All the experiments were repeated thrice and the statistical comparison has been done using Tukey's Multiple comparison test. **Conclusion:** The present study has clearly demonstrated the potency of Methanol extract of *Centella asiatica* (L.) Urban for its antigenotoxic activity and neuroprotective potential.

POS-TUE-249

AGE RELATED CYTOSKELETAL PATHOLOGY IN HUMAN BRAIN: A MORPHO-PATHOLOGICAL COMPARISON BETWEEN SRI LANKAN (COLOMBO) AND INDIAN (KARNATAKA) SAMPLES

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Purpose: Recent studies suggest that much of the dementia prevalence is attributable to rising numbers of people living in low and middle income countries. This led us to perform a comparison between two neighbouring low to middle income populations in Southeast Asia. **Methods:** Age matched elderly brains of 50 Sri Lankans and 42 Indians were subjected to neuropathological screening using histopathological/immunohistochemical techniques. **Results:** In the logistic regression analysis, increasing age was associated significantly on AD related (Braak, CERAD & CAA) pathologies. Population and sex as factors didn't play significantly in AD related changes, however, Sri Lankans showed a higher degree of Braak pathology compared to Indians- Fisher's exact test (p=0.014). Some of the aging changes in the fronto temporal regions-spongiosis [odds ratio (OR)=8.507, 95% CI=2.981-24.279], dilated perivascular spaces (OR=3.878, 95% CI= 1.237-12.154) and neuronal loss in DG (OR=5.884, 95% CI= 1.806-19.167) differed significantly between the populations and were prominent in the Sri Lankan brains. Neuronal loss in CA1 region was associated with age only (OR=1.15, 95% CI= 1.066-1.241). **Conclusion:** Age related pathomorphological changes seem to be more in elderly Sri Lankans compared to elderly Indians.

POS-TUE-250

FIBROBLAST GROWTH FACTOR 13 IS A MICROTUBULE-STABILIZING PROTEIN REGULATING NEURONAL POLARIZATION AND MIGRATION

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Purpose: Secretory fibroblast growth factors (FGFs) and their receptors are known for their regulatory function in the early stages of neural development. FGF13, a nonsecretory protein of the FGF family, is expressed in cerebral cortical neurons during development and is a candidate gene for syndromal and nonspecific forms of X-chromosome-linked mental retardation (XLMR). However, its function during development remains unclear. **Methods:** We use multiple biochemical assays to identify FGF13 as an MSP in cortical neuron. We also use in utero electroporation and knockout mice to investigate the function of FGF13 in neural development. **Results:** We show that FGF13 acts intracellularly as a microtubule-stabilizing protein required for axon and leading process development and neuronal migration in the cerebral cortex. FGF13 is enriched in axonal growth cones and interacts directly with microtubules. Furthermore, FGF13 polymerizes tubulins and stabilizes microtubules. The loss of FGF13 impairs neuronal polarization and increases the branching of axons and leading processes. Genetic deletion of FGF13 in mice results in neuronal migration defects in both the neocortex and the hippocampus. FGF13-deficient mice also exhibit weakened learning and memory, which is correlated to XLMR patients' intellectual disability. **Conclusion:** In this study, FGF13 is identified as an MSP enriched in the growth cones of cortical neurons. Neuronal migration is delayed, and severe mental retardation is found in Fgf13 knockout mice, indicating an essential role of FGF13 in establishing neural circuits in the cerebral cortex and enabling cognitive functions.

POS-TUE-251

ALLOMETRIC VARIATION OF SENSORY BRAIN REGIONS DURING THE ONTOGENY OF THE SOUTHERN HEMISPHERE LAMPREY, GEOTRIA AUSTRALIS

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Lampreys are extant representatives of stem vertebrate forms and can be found in most temperate marine and riverine habitats. They have an anadromous life cycle consisting of a larval (ammocoete) stage, which lives in burrows at the bottom of rivers and is a filter-feeder, and an adult stage, which migrates downstream to enter the sea, where they become parasitic on other fishes, and later return to the rivers to spawn and die. Each stage occupies a different ecological niche, where a differential development of different sensory brain structures is expected. Purpose: We proposed to test whether there are specialised senses associated with each developmental stage by measuring the volume of a range of sensory brain regions. **Methods**: Volumetric estimates of the size of different primary sensory areas were performed at four phases of the life cycle of *Geotria australis* (ammocoetes, n=5; downstream migrants, n=5; upstream migrants, n=6; mature adults, n=4) and subsequently compared. **Results**: All the studied regions develop at a different rate throughout ontogeny; the relative volume of the pineal organ was larger in ammocoetes, whereas the olfactory bulb and visual centres were relatively more developed in adults than in larvae. **Conclusion**: Based on our results, we predict that larvae rely mostly on non-visual cues, while adults have more developed visual and olfactory modalities. We consider this variation is related to changes in light and other environmental signals these animals perceive during their protracted lifecycle.

POS-TUE-252

OREXIN TYPE-1 RECEPTOR MEDIATES THE DEVELOPMENT OF TOLERANCE TO MORPHINE IN LATERAL PARAGIGANTOCELLULARIS NUCLEUS

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Purpose: Orexin is involved in morphine-induced physical dependence and withdrawal. Lateral Paragigantocellularis (LPGi) is a key brain structure implicated in the expression of somatic signs of morphine withdrawal syndrome. Orexin type 1 receptor (OXR1) has been found in LPGi nucleus. In this study the effect of Orexin type 1 receptor blockade on neural activity of LPGi during the development of morphine tolerance was investigated. **Methods:** Male Wistar rats (n=60) weighing 250-300g were used. To incite tolerance, morphine sulfate was injected intraperitonealy (10mg/kg, i.p.) once a day for 6 days (n=13). A selective orexin type 1 receptor antagonist (SB-334867) was microinjected into the right cerebral ventricle (10µg/10µli.c.v.) immediately before morphine injection (n=21). On day 7, the effect of morphine (10mg/kg, i.p.) on LPGi neural activity was studied using in vivo extracellular single unit recording to show the development of tolerance to morphine at the cellular level. **Results:** Morphine injection during 6 days led to the development of tolerance to morphine in LPGi neurons which was observed as a significant decrease in responses of LPGi neurons to morphine. Administration of SB-334867 before each morphine injection reversed responses of LPGi neurons to morphine. **Conclusion:** We showed that orexin type 1 receptor blockade by SB-334867 prevent the development of tolerance to morphine in LPGi neurons. Further studies are required to determine molecular and anatomical mediators which are thought to be involved in this phenomenon.

POS-TUE-253

PREVALENCE OF CSF ALZHEIMER'S DISEASE-LIKE PROFILES IN MIDDLE-AGED HIV+ INDIVIDUALS, RELATIONS TO APOE GENOTYPING AND HIV DISEASE MARKERS

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Purpose: Determine the prevalence of Alzheimer's disease (AD) risk in aging and chronically HIV-infected (HIV+) persons who are successfully treated with combination antiretroviral therapy (cART). **Methods**: 43 adult males and 1 female with stable chronic HIV disease [aged 57, SD=8 years, HIV duration 20 (5-25) years, undetectable plasma and CSF HIV RNA] were enrolled under a prospective observational study. All underwent baseline standard neuropsychological testing, APOE genotyping and a CSF lumbar puncture to assess CSF Aβ1-42, h-tau and p-tau concentrations. Risk for AD was evaluated using published cut-offs. **Results**: These cut-offs were applied to 8 HIV-negative subjects for reference: no elderly controls had a CSF-AD profile; all AD patients had a CSF-AD profile. Of the HIV+ individuals, 11.4% had a CSF-AD profile. Logistic regressions showed that APOE ε4/ε4 (p=0.03) and having previously diagnosed HIV-associated neurocognitive disorder (HAND) (p<0.03) were significantly associated with a CSF-AD like profile. Having a CSF-AD profile was associated with lower current neurocognitive performance (p<.002). **Conclusions**: Some patients with chronic HIV disease have ten-fold higher risk for AD based on CSF biomarkers, relative to the general population of the same age. Known genetic factors for this age group were associated with a CSF-AD like profile, as well as a past HAND diagnosis and current lower neurocognitive functions. Our research argues for renewed research effort to understand the consequences of brain aging in HIV+ persons.

POS-TUE-254

RAPID IMMUNODETECTION OF NEURONAL PROTEIN BIOMARKERS

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Western blotting is a well established technique used for the detection of specific proteins from complex mixtures. Standard immunodetection is a laborious multi-step process requiring more than three hours from membrane blocking to signal detection. Here we present a simplified, yet versatile vacuum-based approach permitting protein detection in less than 30 minutes. The system can be used to expedite routine western blotting, as well as for rapid multiplex protein detection either simultaneously in single blot or by re-probing the same blot multiple times without stripping. Vacuum filtration enables efficient blocking in a few seconds, compresses the antibody binding step(s) to 10 minutes, and further offers a means of rapid blot washing; this single time-saving adaptation enables users to process several blots in a day. The system is fully compatible with standard protocols using common reagents and equipment. This new method was used to evaluate detection of several important neurological markers in human brain tissue lysates (healthy and Alzheimer's). To ensure proficiency in downstream applications, lysates were analyzed for total protein recovery and lipid content using a mid-infrared (MIR) based detection system. Results demonstrated that the new western blotting method and the MIR based quantification systems are compatible and complementary.

POS-TUE-255

ALPHA-SYNUCLEIN TOXICITY TO PRIMARY ADULT RAT CORTICAL NEURONS

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Purpose: The most cytotoxic form of α-synuclein (α-Syn) is the oligomers that form at the beginning of assembly while fibrils are regarded as a storage or inert form presumably less toxic. To further dissect the mechanisms of various α-Syn forms, we have used a primary cortical neuronal culture from adult rates of 7 to 18 month of ages to examine the toxicity of α-Syn and other Parkinsons disease relevant factors. **Methods:** In this study, we investigated the dose responses of adult rat neurons to various concentrations of α-Syn isoforms. Different concentrations (1μM, 5 μM and 10 μM) of α-Syn monomers, oligomers and fibrils were applied to the cortical neurons for 24 h, monitored by propidium iodide and DAPI fluorescence staining. **Results:** We showed that, α-Syn fibrils were significantly more toxic to adult neurons than were oligomers and monomers. Oligomers did show toxicity until the concentration reached 10μM, whereas monomers did not show significant toxicity at all concentrations examined. **Conclusion:** Our result provided the evidence that primary adult neurons are susceptible to α-Syn fibrils.

POS-TUE-256

BINOCULAR RIVALRY: STABILITY VERSUS INSTABILITY

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Introduction. Binocular rivalry, the variable percept resulting from incompatible monocular stimuli, can be induced with intermittent or continuous stimuli. The percept is known to be more stable in the first case than in the second. We aimed to find whether perceptual stability, or the lack of it, is reflected in the psychometric function. Methods. Binocular rivalry was induced in 60 adult human subjects with an oblique grating presented to one eye and an orthogonal grating to the other eye. The two monocular stimuli had the same spatial frequency (3 cycles/deg) and chromaticity (grey), but differed in contrast: the sum of the monocular contrasts equalled 1. In the intermittent case the gratings were presented for 1 s in every 5, with a spatially uniform stimulus in the intervening interval, and subjects signalled their percept after each presentation. During continuous rivalry subjects signalled their percept each time it changed. Results. After removing mixed percepts from the data, a psychometric function – the probability of seeing the right-eye stimulus versus the contrast of that stimulus – was calculated for both types of experiment. The psychometric function was similarly biased in the two cases, in that the left eye's contrast had to be higher than the right eye's contrast to obtain equal probabilities of the two percepts. We take this to indicate a bias to the right eye regardless of perceptual processing. The psychometric functions differed, however, in that the slope during continuous rivalry was about half of that during intermittent stimulation. This shows that neural noise plays a greater role in percept selection during continuous rivalry. Conclusions. Perceptual instability is greater during continuous rivalry than during intermittent rivalry, and this instability is reflected in the decreased slope of the psychometric function.

POS-TUE-257

MTORC1 INHIBITION REDUCES MOTIVATION TO CONSUME COCAINE

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Purpose: The mammalian target of rapamycin (mTOR), a serine threonine protein kinase, together with Raptor forms a multiprotein complex termed mTORC1. Rapamycin (RAPA), a specific mTORC1 inhibitor, reduces alcohol drinking, but not cocaine self-administration, under low effort conditions. We found that intra-peritoneal (i.p.) RAPA reduced responding for cocaine when assessed using progressive ratio (PR), however these effects were associated with reduced weight gain and locomotor activity. Therefore, we assessed the effect of intra-cerebroventricular (i.c.v.) injections of RAPA on PR responding for cocaine, locomotor activity, weight gain and readouts of mTORC1 activity in the nucleus accumbens (NAC). Methods: Sprague-Dawley rats (250-300g) were trained to self-administer cocaine (FR1-5) and then tested on a PR schedule for three days. Three hours prior to PR testing, rats received either 0 or 25ug RAPA (i.c.v; n=8/group). Animals were sacrificed 24h after PR testing and the NAC dissected and processed for western blot analysis. A second group of animals were tested for effects of RAPA on locomotor activity. Results: i.c.v. RAPA treatment significantly suppressed PR breakpoints ($p<0.05$) and reduced phospho-p70s6K and GluR1 AMPA receptor subunit levels ($p's<0.05$). There was no change in locomotor activity across treatment and weight loss was less pronounced than that observed following systemic RAPA. Conclusions: Suppression of central mTORC1 activity leads to a reduction in motivation to consume cocaine under conditions that require increasing effort with limited effects on locomotor activity or bodyweight. Together these data indicate that mTORC1 may control the dynamic regulation of synaptic proteins within the NAC that are required for the expression and maintenance of drug reward.

POS-TUE-258

K3 MICE OVEREXPRESSING MUTATED TAU IN SUBSTANTIA NIGRA HAVE IMPAIRED ABILITY TO DEVELOP HABITS

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Purpose: A prominent cognitive impairment seen in Parkinson's disease (PD) patients is the decreased ability to retain, develop and perform habitual actions, while the goal-directed actions remain intact. The most common approach to generate a loss of striatal dopaminergic innervation is by lesioning the dopamine neurons in the substantia nigra by using 6-hydroxydopamine. Although efficient, this model doesn't provide a progressive decline of striatal dopaminergic innervation seen in PD and thus can't be used as a model to study the early and late phases of the disease. Therefore, we are currently investigating the recent transgenic K3 mice, where mutated human tau is over-expressed in neurons of the substantia nigra resulting in decreased striatal dopamine levels and parkinsonian symptoms such as tremor, bradykinesia, abnormal gait, and postural instability. In the current model we investigate the ability of the K3 mice to obtain and express goal-directed and habitual actions. **Methods:** Animals were trained to press a lever to obtain purified pellets and trained to promote habitual behaviour using a random interval 60s schedule. The training was carried out for 9 days before investigating the animal's sensitivity to outcome devaluation by allowing them to feed to satiety with purified pellets or chow, followed by a 10min lever extinction test. **Results:** Compared to its wild-type littermates (n=6), the K3 mice (n=9) where still sensitive to outcome devaluation upon overtraining, indicating that they have an impaired ability to develop habitual behavior. **Conclusion:** The K3 mice represent a potentially useful cognitive model of PD, which provides us with an opportunity to study the impaired ability to retain, develop and perform habitual actions during the progression of the disease.

POS-TUE-259

CHARACTERISING THE EFFECTS OF IMPACT VELOCITY AND ANIMAL BIOMETRICS ON BRAIN AND BEHAVIOUR IN AN IMPACT INJURY MODEL

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Introduction: The brain damage and behavioural deficits caused by impact injury in real-life car and sporting accidents depend on factors such as the impact velocity and the person's biometrics, most pertinently the weight and age. This study was designed to understand how variations in impact velocity and animal biometrics cause variations in behavioural deficits and brain damage using a well-established weight-drop impact acceleration (WDIA) animal model of traumatic brain injury. **Methods:** A narrow range of impact velocities of 5.4 m/s, 5.85 m/s and 6.15 m/s (measured by high speed video recording (2000 frames/second)) were used to generate diffuse brain injury on adult rats (~350 grams, n=9-14/group). Behaviour was assessed using two sensorimotor tasks for balancing, movement and coordination controls. Diffuse brain injury of axonal swelling and bulbs was demonstrated by immunohistochemistry for β-amyloid precursor protein and neurofilament heavy-chain. **Results:** As impact velocity increased from 5.4 to 5.85 to 6.15 m/s there was a systematic increase in morality and in behavioural deficits. Performance on the rotarod task decreased significantly from a pre-injury 29.3±03 rpm to 13.5±2.9 rpm in 6.15 m/s group, while beam walking deficit was significantly increased. Axonal injuries were minimal or absent at the velocity of 5.4 and 5.85 m/s and massively increased in axonal swelling and bulbs were detected at 6.15 m/s. Higher impact acceleration results more severe brain damage. Furthermore, age played a major role in mortality, but that weight per se (when age and impact velocity are controlled) had no effect. **Conclusion:** Our study has been specifically designed to study how different levels of impact velocity and prime animal biometrics affect the injury severity. It has better defined the relationship between impact velocity and behavioural deficits and axonal damage in this model in a way that allows translation to humans in accidents.

POS-TUE-260

CHARACTERISING NEUROINFLAMMATION IN A PRE-CLINICAL MODEL OF PARKINSON'S DISEASE

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There is much evidence suggesting that microglia play a role in the characteristic dopaminergic degeneration in Parkinson's disease (PD). While the activation of microglia is rapid, up to 70% of dopaminergic neurons may have degenerated before parkinsonian symptoms appear. Commonly used rodent models of PD create large lesions, mimicking dopaminergic degeneration seen at the end of the disease, which provides little detail of what is occurring pre-clinically. Purpose: We used a graded version of the 6-OHDA rat model of PD to correlate parkinsonian behaviour with immunohistochemical data of changes in glial cells and dopaminergic neurons of the Substantia Nigra Pars Compacta (SNc). Methods: Sprague Dawley rats (n=60) underwent baseline testing for movement behaviour prior to receiving graded 6-OHDA lesions to the medial forebrain bundle (control n=12, mild lesion n=16, moderate n=16, large n=16). Post lesion behavioural testing was recorded and brain tissue prepared for immunohistochemical analysis of OX-42 and GFAP expression. Results: 6-OHDA lesions resulted in a significant increase in OX-42 expression ($P<0.05$), and GFAP expression ($P<0.05$) in the SNc of the lesion side when compared to the non-lesion side. This was not observed in controls. The expression of these proteins was both dose and time-course dependent. Results were correlated with abnormal movement behaviours in the lesion rats. Conclusion: We demonstrated both time-course and lesion size-dependent activation of microglia in the SNc and provide evidence for the early and ongoing activation of microglia.

POS-TUE-261

DOES NEONATAL RESUSCITATION WITH A SUSTAINED INFLATION CAUSE CEREBRAL VASCULAR INJURY IN ASPHYXIATED LATE PRETERM LAMBS?

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Purpose: Successful resuscitation of asphyxiated neonates immediately after birth requires adequate ventilation. We have shown that a single sustained inflation (SI) of 30s improves circulatory recovery in late preterm asphyxiated lambs[1], and rapidly increases cerebral blood flow but concern remains about possible adverse effects. Here we aim to determine the effect of different SI strategies on cerebral vascular integrity in asphyxiated late preterm lambs. Method: Lambs were delivered and instrumented at 139±2 days gestation (term ~147 days gestation). Birth asphyxia was induced by delaying ventilation after cord clamping by ~10min and then lambs were randomised to receive 5 consecutive 3s SIs (n=6), a single 30s SI (n=6) or no SI (n=6); all followed by mechanical ventilation (30min). Sections from cerebral hemispheres were immunostained with anti-sheep serum to score vascular leakage. Groups were compared by Kruskal-Wallis ANOVA on ranks. Data expressed as median, 25-75% percentile. Results: Vascular leakage scores in the cortical grey matter (5x3s SI: 0, 0-0.7%; 30s SI: 0.5, 0-0.8%; no SI: 0, 0-0.3%), periventricular white matter (5x3s SI: 0, 0-1.1%; 30s SI: 0, 0-2.0%; no SI: 0, 0-0.4%) and subcortical white matter (5x3s SI: 0.5, 0-1.2%; 30s SI: 1.0, 0.5-2.0%; no SI: 0.5, 0-1.1%) were similar in lambs that received a SI (5x3s and 30s) compared to lambs that received no SI. Conclusions: Resuscitation with different SI strategies did not exacerbate blood brain barrier permeability compared to lambs with no SI. Therefore a SI for immediate neonatal resuscitation merits further consideration. [1] Klingenberg et al, Arch Dis Child Fetal Neonatal Ed (2012).

POS-TUE-262

CHANGES IN DEFAULT-MODE NETWORK DURING SHIFTING RESPONSE-SET IN HUNTINGTON'S DISEASE: 30 MONTH LONGITUDINAL DATA FROM THE IMAGE-HD STUDY

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Cognitive flexibility, the executive capacity to adaptively shift attention among stimuli or responses according to context, has been shown to be affected in Huntington's disease (HD). Specifically, premanifest (pre-HD) and symptomatic (symp-HD) individuals are variously impaired in delayed alternation tasks, shifting between phonemic clusters during word-list generation, cued word finding tasks, and tasks requiring shifting across perceptual categories or dimensions.**Purpose**: We aimed to characterize, for the first time, the linear progression of brain activation over three time points during a shifting response-set task in pre-HD and symp-HD participants, relative to controls. **Methods**: Participants were recruited as part of the IMAGE-HD study (a longitudinal multimodal imaging study) and were assessed at baseline, 18 and 30 months. Data for a total of 60 participants, who performed at ≥70% accuracy, were included in the analyses (11 symp-HD, 26 pre-HD and 23 controls). **Results**: We found a significant group-by-time interaction in two regions of the default-mode network (DMN): the medial prefrontal and posterior cingulate cortices. Linear decreased activity over time in these regions significantly differed across groups, with symp-HD exhibiting the greatest decrease followed by pre-HD and controls. **Conclusion**: Greater DMN deactivation across time in HD may reflect a compensatory response where higher disease-related costs associated with attention shifting are met by a relocation of resources from self-referential to goal-directed networks.

POS-TUE-263

THE DYNAMIC ACTION POTENTIAL CLAMP AS A TOOL FOR SCREENING ANTI-EPILEPTIC DRUGS

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Purpose: The majority of anti-epileptic drugs (AEDs) appear to act on voltage-gated sodium channels (VGSC) that are central to action potential (AP) generation. Current in vitro methods of assessing the efficacy of prospective AEDs on VGSCs are limited to a range of 'static' electrophysiological protocols that may not fully account for the dynamic state a channel experiences during an AP. In this study we develop a novel method, the dynamic action potential clamp (dAPC) which uses AP firing rate as quantifiable outputs, enabling the rapid assessment of prospective AEDs providing a potentially more predictive output over standard electrophysiological assays. **Methods:** HEK293t cells transfected to express NaV1.4 voltage-gated sodium channels were recorded in voltage-clamp mode and electrically coupled with a real time computer simulation of a single cell compartment. This created a real time feedback loop between the computer model and the VGSC channels in the HEK cell. The computer simulation ran Hodgkin-Huxley type models of leak and potassium conductance while the sodium component of the membrane current was provided by the HEK cell. 500ms current steps (50-300pA) were delivered through the dAPC and AP firing rate was measured at different concentrations of the AED carbamazepine (CBZ) that acts through VGSCs. **Results:** CBZ exposure suppressed higher frequency action potential firing (n=8 cells) but permitted lower frequency firing. **Conclusion:** We were able to demonstrate that CBZ only blocks high frequencies of action potential firing providing proof-of-principle that dAPC can predict AED efficacy.