POSTERS
POS-MON-001

ORAI1 IS NECESSARY FOR GROWTH CONE TURNING

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The foundations of the nervous system circuitry are established in early development as young neurons send out axons to connect with target cells. The accuracy of axon guidance is dependent on the navigational structure at the distal tip of the extending axon, the growth cone. Growth cone motility is dependent on changes in intracellular calcium levels. A key source of calcium within growth cones is store operated calcium entry (SOCE). The stromal interaction molecule STIM1 activates SOCE upon depletion of calcium from the endoplasmic reticulum and we have reported that STIM1 mediated SOCE is necessary for growth cone navigation. In order to activate SOCE, STIM1 has been reported to interact with several proteins on the plasma membrane including the Orai proteins and transient receptor potential canonical (TRPC) channels. However, whether STIM1 activates Orai and/or TRPC proteins within growth cones is unknown. We have examined Orai protein distribution within the developing and adult nervous system and found discrete sub-cellular localisation. We used siRNA to reduce expression of Orai1, or Orai2 in embryonic rat sensory neurons and tested whether growth cone function was perturbed in a growth cone turning assay. We found that reduced expression of Orai1 but not Orai2 abolished growth cone turning in response to Netrin-1 (-2.33 ± 1.51°; p=0.001). Similarly, Orai1, but not Orai2 was also required for growth cone turning away from Sema-3a (-0.46 ± 1.77°; p<0.05). These data suggest that Orai1 forms the STIM1-mediated SOCE channel in navigating growth cones.


POS-MON-002

ADOLESCENT TESTOSTERONE, BDNF AND TRKB IN THE PRIMATE FRONTAL CORTEX AND HIPPOCAMPUS

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Purpose: We have recently shown, in contrast to adult brain, circulating adolescent testosterone reduces cell survival in primate hippocampus, suggesting differential effects of testosterone on the brain at adolescence compared to adulthood. Brain derived neurotrophic factor (BDNF), acting through TrkB receptor, is important for neuronal growth and differentiation. Testosterone modulates BDNF expression in the adult rodent brain; it is unknown, however, how testosterone impacts growth factors in primate adolescence. We examined the interaction between adolescent testosterone, BDNF and TrkB in frontal cortex (FC) and hippocampus; and the relationship between testosterone, BDNF, TrkB, and hippocampal neurogenesis in male rhesus macaques. Methods: Prepubertal macaques (2.5 years) were gonadectomised (n=6) or sham-operated (n=6) and sacrificed at 4.5 years. BDNF and TrkB mRNA expression were quantified in hippocampus and FC using in situ hybridization and RT-PCR. Protein expression was measured in FC by immunoblotting. Results: In FC, gonadectomy increased full length TrkB (TrkBTK+) protein compared to the intact group (p=0.03). There was a trend to increased mature BDNF protein in the gonadectomised group (p=0.07), BDNF IV-IX mRNA expression showed a negative correlation with PM testosterone (p=0.04, r=-0.83). Gonadectomy did not change hippocampal TrkB and BDNF expression. There was a trend for a positive correlation between TrkBTK+ mRNA expression and cell proliferation (Ki67+ cells/mm3) in the dentate gyrus (p=0.07, r=0.57). Conclusion: Adolescent testosterone negatively regulates full-length TrkB and BDNF expression in FC PFC; as such, adolescent primate FC may be more responsive to testosterone than hippocampus. Our results suggest full length TrkB receptor expression may be linked to cell proliferation, but this may be independent of circulating testosterone levels.

POS-MON-003

THE TRANSTHYRETIN GENE IS EXPRESSED IN HUMAN OLIGODENDROCYTE CELLS

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Purpose: Transthyretin (TTR) is known as a thyroid hormone distributor protein that is mainly synthesised in the liver and in the choroid plexus of the brain. It distributes thyroid hormones around the body via the bloodstream, and from the blood to the cerebrospinal fluid to reach the parenchymal cells of the brain. The purpose of this study was to investigate the expression of the TTR gene in differentiated and undifferentiated human oligodendrocyte precursor cells (hOPCs).

Methods: hOPCs were derived from NIH approved H9 human embryonic stem cells (hESC) following their culture and differentiation conditions. Four main techniques have been used to examine the expression of the TTR gene within the prominent of hOPC population upon differentiation, suggesting differential effects of testosterone on the brain at adolescence compared to adulthood. Brain derived neurotrophic factor (BDNF), acting through TrkB receptor, is important for neuronal growth and differentiation. Testosterone modulates BDNF expression in the adult rodent brain; it is unknown, however, how testosterone impacts growth factors in primate adolescence. We examined the interaction between adolescent testosterone, BDNF and TrkB in frontal cortex (FC) and hippocampus; and the relationship between testosterone, BDNF, TrkB, and hippocampal neurogenesis in male rhesus macaques. Methods: Prepubertal macaques (2.5 years) were gonadectomised (n=6) or sham-operated (n=6) and sacrificed at 4.5 years. BDNF and TrkB mRNA expression were quantified in hippocampus and FC using in situ hybridization and RT-PCR. Protein expression was measured in FC by immunoblotting. Results: In FC, gonadectomy increased full length TrkB (TrkBTK+) protein compared to the intact group (p=0.03). There was a trend to increased mature BDNF protein in the gonadectomised group (p=0.07), BDNF IV-IX mRNA expression showed a negative correlation with PM testosterone (p=0.04, r=-0.83). Gonadectomy did not change hippocampal TrkB and BDNF expression. There was a trend for a positive correlation between TrkBTK+ mRNA expression and cell proliferation (Ki67+ cells/mm3) in the dentate gyrus (p=0.07, r=0.57). Conclusion: Adolescent testosterone negatively regulates full-length TrkB and BDNF expression in FC PFC; as such, adolescent primate FC may be more responsive to testosterone than hippocampus. Our results suggest full length TrkB receptor expression may be linked to cell proliferation, but this may be independent of circulating testosterone levels.

Methods: hOPCs were derived from NIH approved H9 human embryonic stem cells (hESC) following their culture and differentiation conditions. Four main techniques have been used to examine the expression of the TTR gene within the prominent of hOPC population upon differentiation, which are reverse transcription polymerase chain reaction (RT-PCR), PCR product sequencing, western blot and immunohistochemical analysis. A12 well plates for immunocytochemistry were performed, with two of these being controls for TTR. Also, RNA for RT-PCR was extracted from two T25 flasks, with 500,000 cells in each, with and without differentiation medium. Finally, protein for Western blot was. Extracted from 500,000 cells in a T25 flask without differentiation medium. Results: TTR mRNA and protein were identified in cultured hOPCs and may relate to a novel function of the TTR within these cells. Conclusion: Our results currently suggest that hOPCs have the ability to synthesize TTR. Such data may lead to the identification of a novel function for TTR related to oligodendrocyte biology.

POS-MON-004

GENETIC MODIFICATION OF REST LEADS TO IN VITRO DEREPRESSION OF NEURONAL GENES DURING NEUROGENESIS

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Background: Rest (RE1 silencing transcription factor, also called NRSF) is involved in the maintenance of the undifferentiated state of neuronal stem/progenitor cells in vitro by preventing precocious expression of neuronal genes. However, the function of Rest during neurogenesis in vivo remains to be elucidated because of the early embryonic lethal phenotype of the conventional Rest knockout mice. Purpose: We have generated Rest conditional knockout mice as well as Rest conditional overexpression mice, which allow the in vivo examination of the genetic modification of Rest during embryonic neurogenesis. Results: Conditional ablation of the exon 4 in Rest, which encode the CoRest binding site, in developing embryos results in embryonic lethality (n=6) like conventional Rest knockout mice. Genetic ablation of Rest in mouse embryonic fibroblasts (n=6) in vivo as well as limb tissue (n=4) in vivo lead to the increased expression of Syt4, Tubb3 and Calb1, which are targets of the Rest repressor complex. Genetic ablation of Rest in neurospheres under the differentiation condition (n=6) in vitro also increased the expression of Syt4 and Calb1 as well as Tubb3. Rest regulates neuronal genes and has no effect in brain tissues (n=4) in vivo and Gfap-positive gial cells. Genetic ablation of Rest during the early neural development by using the Sox1-Cre promoter again does not cause any detectable abnormality at different developmental stages and ages in vivo (n=6). Furthermore, conditional overexpression of Rest also did not show any significant difference in vivo (n=6). Conclusion: Rest plays a role in suppressing the expression of neuronal genes, but Rest is dispensable for embryonic neurogenesis such as the maintenance and differentiation of neuronal progenitor cells in vivo.
UMBILICAL CORD BLOOD CELLS AS A POTENTIAL TREATMENT FOR ASPHYXIAL BRAIN INJURY

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Background: Birth asphyxia can result in neurological damage manifesting as cerebral palsy in surviving infants. By reducing inflammation, umbilical cord blood (UCB) cells could ameliorate neuropathology caused by birth asphyxia. Methods: Birth asphyxia was induced via umbilical cord occlusion in term lambs. Umbilical cord blood was obtained at time of delivery. UCB cells were isolated from the buffy coat and labelled with fluorescing iron label. Autologous UCB cells (100×10^3) were administered to a subset of UCO lambs 12 hours after birth. Lamb behaviours were monitored and at 12 and 72h after birth we undertook magnetic resonance spectroscopy (MRS) to assess the ratio of brain metabolites N-acetyl aspartate (NAA) to. At 72 hours, lambs were euthanased. Results: Asphyxia+UCB cell lambs showed improved muscle tone and suckling responses, compared to asphyxia lambs. NAA/lactate was significantly increased in asphyxia lambs (n=12) compared to control lambs (n=8) at 12 h (2.0±0.6 vs 0.7±0.07) and 72 hours (0.3±0.19 vs 0.1±0.06), in asphyxia+UCB cell lambs NAA/lactate was returned to basal at 72 h (0.9±0.02); 48h after UCB administration. Fluorescing UCB cells were found in the cortex, white matter and deep grey matter of asphyxia+UCB cell animals. Neuronal cell death (creatinined/acidicus staining), was significantly increased in the hippocampus of asphyxia brains (24.4±4.6cells/mm^3/mm^3) compared to controls (2.0±1.4cells/mm^3/mm^3); while asphyxia+UCB cell lambs (7.7±3.1cells/mm^3/mm^3) were not significantly different from controls. Conclusion: Administration of autologous umbilical cord blood cells to newborn lambs following birth asphyxia improves behavioural outcomes, reduces injurious brain metabolites, and reduces neuronal cell death. Cord blood cells show potential for treatment of birth asphyxia-brain injury in newborns.

Does High Dose Caffeine Exposure Affect the Developing Cerebral Cortex?

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Purpose: Caffeine is used to treat apnoea in preterm infants. The efficacy using standard caffeine doses is modest, thus there is pressure on clinicians to administer higher doses. However, the effects of higher doses of caffeine administration on the very immature brain have not been thoroughly investigated. We have previously demonstrated that high dose caffeine exposure leads to an increase in brain weight with no effects on the white matter in the very immature ovine brain. Here we investigate whether high dose caffeine exposure injures the cerebral cortex. Methods: Caffeine (50mg/kg loading; 40mg/kg daily maintenance dose; n=9) or saline (n=9) was administered to the ovine fetus via the maternal circulation [104-118 days of gestation (DG); term = 147 DG]. Fetal and maternal plasma caffeine concentrations were determined. At 119 DG the cerebral cortex was immunostained to identify microglia (Iba-1) and apoptotic cells (TUNEL). Results: Fetal plasma caffeine concentrations peaked at ~30mg/L, 1 hour post caffeine administration and reached a concentration of ~20mg/L 6 hours after administration. There was no difference (p>0.05) between caffeine-exposed and control fetuses in cerebral grey matter volume or in the overall density of Iba-1-positive microglia (resting or activated) or TUNEL-positive apoptotic cells. Conclusions: High dose caffeine administration does not appear to affect cortical growth; nor does it result in microgliosis or increased apoptosis. The effects of high dose caffeine exposure on other components of the cerebral cortex needs to be investigated.
INTEGRATION OF TRANPLANTED INTERNEURONS IN THE EMBRYONIC AND POSTNATAL CORTEX
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**Purpose:**
The increased susceptibility of subtype- and laminar-specific loss of cortical interneurons in neurodevelopmental disorders emphasizes the importance of microcircuits as a causal factor in disease progression. Transplantation of embryonic-derived interneuron progenitors into the postnatal cerebral cortex in rodents has been shown to increase the inhibitory tone, but it is still unclear whether a time-window exists where interneurons can be incorporated into the host cortex and whether this integration occurs with subtype and laminar specificity.

**Methods:**
Donor interneuron progenitors were dissociated from GAD67-knockin-GFP medial ganglionic eminence at mid-corticogenesis [embryonic day (E) 14.5] and injected in utero into the lateral ventricle of a wild-type E15.5 host cortex. For postnatal transplantations, the same stage donor cells were injected into a wild-type postnatal day (P) 4 host cortex at the following coordinates from bregma: 0.7mm A, 2.0mm L, 1.2mm D. Each group contains a minimal of n=3 independent transplantations.

**Results:**
Interneurons born at E14.5 during normal development are destined to reside in cortical layer IV and were used as a control of laminar positioning and specification. By assessing the position of the transplanted GFP-positive interneurons into the embryonic brain, these donor cells align with normal development in positioning and specification into parvalbumin or somatostatin-positive interneurons. In contrast, laminar position is altered when progenitors were transplanted into the postnatal cortex with reduced integration in layer IV but enhanced accumulation in lower layer VI.

**Conclusion:**
Our results highlight that the maturation stage of the host cortex can influence the positioning of transplanted interneurons. This has underlying implications on our understanding of interneuron maturation and the use of cell transplantations to alter cortical plasticity and neural activity.

THE ROSTRAL MIGRATORY STREAM RECAPITULATES DOPAMINERGIC INTERNEURONS OF THE ADULT OLFATORY SYSTEM
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**Background:**
The demonstration of adult brain neurogenesis strengthens the rationale for regenerative therapies for neurodevelopmental disorders. The ‘ventriculo-olfactory neurogenic system’ (VONS) provides a window into adult dopaminergic neurogenesis, where we previously described SVZ neuroblasts recapitulating a population of dopaminergic neurons within the olfactory bulb (OB). We have also observed showing modulation of cell proliferation and migration within the VONS.

**Aims:**
Here we examine evidence for dopaminergic cell death and the directed differentiation of SVZ neuroblasts into the dopaminergic phenotype. Methods: We focally ablate the olfactory epithelium (OE) over an extended period (4 days) in adult mice (n=5). The thymidine analogue EdU was used to tag newly generated cells for quantification, which co-localised with a panel of key proteins markers to define their differentiation. Results: Extended lesioning of the olfactory receptor neurons causes a decrease in glial cell activation. The significant decrease in type 1 periglomerular neurons expressing TH was partially driven by cleaved-caspase3 mediated mechanisms. Dopamine transporter, dopa-decarboxylase and vesicular monoamine transporter immunostaining failed to identify relevant olfactory interneurons. An increased intensity in cell proliferation of the SVZ following the focal ablation of the olfactory sensory neurons and an increase in the recruitment into the periglomerular dopamine cell phenotype in the OB was defined using EdU labelling. Conclusions: This data suggest the olfactory sensory innervation and or the loss of the innervation modulates cell genesis in the SVZ and recapitulation of the dopaminergic periglomerular interneurons in the olfactory bulb. The study of the modulation of dopaminergic neurogenesis in the adult brain is of significance for investigations on Parkinson’s disease therapy.

ISOULATION OF MIDBRAIN DOPAMINE PROGENITORS FOR TRANSPLANTATION IN PARKINSON’S DISEASE
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**Purpose:**
Parkinson’s disease (PD) is characterised by the progressive and irreversible degeneration of midbrain dopamine (mDA) neurons. Clinical trials using foetal tissue grafts have provided proof-of-principle that cell transplantation therapy can be remarkably effective as a treatment for PD. The use of foetal tissue, however, imposes significant limitations as a realistic mainstream therapy. The future of cell-based therapies for PD is almost certainly dependent on the development of safe and effective procedures using stem cells as an alternative source of mDA neurons for use in transplantation procedures.

**Methods:**
To standardise stem cell preparations prior to transplantation we have used immunological selection techniques to selectively label and isolate the transplantable mDA neurons from foetal and cultured cells utilizing fluorescence activated cell sorting (FACS). Candidate proteins for sorting were identified through microarray analysis of mDA neurons in foetal tissue, generating a list of genes coding for cell surface proteins uniquely expressed in the transplantable mDA cell population.

**Results:**
Foetal and mouse embryonic stem cells sorted using the candidate proteins were successfully transplanted into rodent models of PD, providing an enriched mDA neuron population capable of structurally and functionally integrating in the in-vivo dopamine depleted brain.

**Conclusion:**
This provides proof of principle that immunogenic sorting is a viable approach for isolating transplantable dopamine neurons and has important implications for the standardisation of stem cell populations.

PHENOTYPE-SPECIFIC NEURONAL DIFFERENTIATION OF HUMAN INDUCED NEURAL PRECURSOR CELLS
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**Purpose:**
We have demonstrated the ability to directly reprogram human adult dermal fibroblasts (HDFs) to neural precursor cells and subsequently functionally mature neurons with the capacity to generate action potentials. This was achieved by transient ectopic expression of the neural genes Sox2 and Pax6 through non-viral plasmid transfection. This exciting technology has wide-reaching applications for future research in the treatment and understanding of neurological disorders through cell-line disease modeling and drug development. To attain this, it is necessary to obtain high yields of specific neuronal phenotypes affected by neurological disorders including Parkinson’s disease and Huntington’s disease. We have therefore optimized protocols for the efficient generation of dopaminergic and GABAergic neuronal cultures using human induced neural precursor (iNP) cells. Methods: Non-viral plasmid transfection was used to transiently over-express Sox2 and Pax6 in adult HDFs. The cells were cultured until colony formation was observed. Colonies were replated and exposed to temporal combinations of differentiation factors including sonic hedgehog, FGF8, BDNF, GDNF and TGFβ3. Neuronal phenotype was determined by immunocytochemistry using markers of dopaminergic neurons (tyrosine hydroxylase), GABAergic neurons (DARRP32 and calbindin) and neurons (TUJ1 and NSE).

**Results:**
Following a range of trials, optimal protocols were established for the generation of dopaminergic and GABAergic neurons. Analysis of two independent lines demonstrated a considerable increase in the number of dopaminergic and GABAergic neurons generated from human iNP cells (50-70%) compared to that reported for induced pluripotent stem cells (10-30%). Conclusions: Adult human-derived iNP cells can generate high yields of dopaminergic and GABAergic neurons providing an efficient cell source for neurological disease modeling and drug development.
POS-MON-013

THE LONG-TERM EFFECTS OF ADOLESCENT INHALANT ABUSE ON GROWTH AND MATURATION IN RODENTS
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Purpose: The abuse of inhaled vapours containing toluene is a significant public health concern, especially in adolescent populations where it coincides with periods of rapid growth and maturation. However, our understanding of the effects on maturation is limited especially with respect to the concentration being inhaled and the frequency of use.

Methods: Male adolescent Wistar rats were exposed to either air or chronic intermittent toluene (CIT) for 1hr for 4 weeks at either 3,000ppm-5xweek or 10,000ppm-3xweek (n=6-12 per group). We characterized the effects of CIT on growth and maturation during the exposure period and following 2 months of abstinence.

Results: Adolescent CIT exposure resulted in a dose-dependent suppression in weight gain due mainly to a reduced percentage of body fat (p<0.05), the effects of which are maintained in abstinence (p<0.05). After the last exposure to CIT kidney and liver, but not brain, weight were reduced in both groups relative to controls (p<0.05) however this recovered following abstinence. Liver function (alcohol dehydrogenase activity) was increased following CIT but only in animals exposed to 10,000ppm (p<0.05). During the exposure period the ratio of water-to-food consumption was increased in CIT animals, but was decreased during abstinence (p<0.05). This was not due to anhedonia (saccharin preference) or altered fluid homeostasis/renal function (rehydration after water deprivation).

Conclusion: Exposure to CIT during adolescence has long-term effects on growth and development, the effects of which were more prominent in animals exposed less frequently but to a higher concentration. Future investigations of the factors mediating altered maturational trajectories induced by CIT are needed.

POS-MON-014

ROLE OF LYSOPHOSPHATIDIC ACID IN THE MAINTENANCE AND DIFFERENTIATION OF HUMAN NEURAL STEM/PROGENITOR CELLS
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Lysosphosphatidic acid (LPA) is a signalling phospholipid that plays broad roles within the nervous system during development and physiopathological conditions. How LPA functions in the human CNS is poorly understood.

Purpose: Here, we studied the effects of LPA on neural differentiation of human pluripotent stem cells. Method: We used human embryonic stem cells (hESCs) (n=3) and human induced pluripotent stem cells (iPSCs) (n>3) and differentiated them towards neural stem/progenitor cells (NS/PCs), astrocytes and early neurons to assess LPA's effect at these various stages. Results: Using hESCs and iPSCs, we demonstrated a similar mRNA profile of expression of LPA receptors upon neural differentiation. We also identified that LPA inhibits NS/PC maintenance as neurospheres by increasing apoptosis in a Rho/ Rho associated kinase (ROCK)-dependent mechanism. Furthermore, we demonstrated that LPA inhibits the neuronal differentiation of iPSCs through the Rho/ROCK and PI3K/Akt pathways, as already observed in hESCs. Lastly, LPA induced neurite retraction of NS/PC-derived early neurons through the activation of the Rho/ROCK pathway. Conclusion: As LPA levels increase in the CNS following inflammation and injury, LPA-mediated inhibition of NS/PC maintenance, neuronal differentiation and neurite retraction may contribute to the poor neurogenesis observed in the CNS following injury. This study also verified the consistency of hESCs and human iPSCs responses to LPA, making them an in vitro tool to study further roles of LPA signalling on human CNS populations. Finally, we assessed LPA effects during neural development and regeneration of zebrafish, allowing a further characterization of LPA signalling biology in homeostasis and pathological condition of CNS.

POS-MON-015

SYMPATHETIC RE-INNERRATION OF THE RAT UTERUS IN THE FIRST 2 WEEKS POST-PARTUM
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Purpose: We have previously found that the pregnant rat uterus near-term is almost completely denervated. Post-partum regrowth of uterine axons is not well-characterized. Here, we examined uterine sympathetic re-innervation during the first 2 weeks post-partum using whole mount preparations.

Methods: Rat uterine horns from post-partum days 1 (P1), 3 (P3), 5 (P5), 7 (P7), 10 (P10), 14 and 14 (P14, n=3) were fixed, stained for tyrosine hydroxylase (TH) using an immunoperoxidase procedure, dehydrated, embedded in resin (P14, n=3) were fixed, stained for tyrosine hydroxylase (TH) using an immunoperoxidase procedure, dehydrated, embedded in resin and mounted on slides.

Results: At P1, sympathetic axons were just beginning to grow back into the uterus through the mesometrium. At P1 and P3, very few TH-immunoreactive axons occurred in the muscle and around blood vessels and many showed growth cones. Innervation density was higher at P3 than at P1. At P5, blood vessels were moderately innervated but few axons were present in the muscle. Axons with growth cones were at maximum density at P3 and P5, occurring around the uterine circumference and on both muscular and perivascular axons. At P7 and P10, blood vessels were significantly innervated and the density of TH-immunoreactive axons in the muscle was low to moderate. TH axons were first found in the linea uteri at P10. At P14, almost all blood vessels were densely innervated and many TH-immunoreactive axons were present in the muscle. By P14, only a few mainly perivascular axons had growth cones.

Conclusion: From almost complete denervation near-term, uterine sympathetic innervation increases progressively from P1 to P14. At P14, the muscle is less densely innervated and blood vessels are slightly less densely innervated than in the non-pregnant state.

POS-MON-016

NDFIP1 REGULATES NEURONAL DEVELOPMENT AND FUNCTION VIA THE MAP-KINASE PATHWAY
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Receptor tyrosine kinase (RTK) signaling pathways are vital for neuronal development and function. Purpose: In this study, we investigate the role of ubiquitination pathways involving the Nedd4 E3 ligase in transmitting growth factor receptor signaling. This process is mediated by Ndfip1, an adaptor and activator of Nedd4 function. Results: Specifically, Ndpif1 regulates Sprouty 2 (Spry2) by ubiquitination leading to reduction of Spry2 and consequently increasing pErk following EGF receptor (EGF-R) activation. In the developing cortex, Spry2 is co-expressed with Ndfip1 in similar cells; and deletion of Ndfip1 results in Spry2 upregulation in these cells. Conversely, overexpression of Ndfip1 in a neuronal cell line (SY5Y cells) results in reduction of Spry2, suggesting that Ndfip1 can regulate Spry2 levels. Conclusion: These results point to Ndfip1/Nedd4 ubiquitination as an important mechanism for gating pErk signaling in the EGF-R pathway.
POS-MON-017

FGF MEDIATES GLIA BRIDGE FORMATION AFTER SPINAL CORD INJURY IN ZEBRAFISH AND MOUSE

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A major barrier to axonal regeneration after spinal cord injury (SCI) is glial scar formation as a result of the astrocytic response to injury. Purpose: Study mechanisms involved in gliogenesis that may facilitate radial glia enrichment at the lesion site rather than scar formation. Methods: Glia proliferation and morphology was examined at the lesion site in different zebrafish transgenic lines in which there is either a loss or gain of Fgf signalling or in Fgf2 treated mice after SCI. Results: Zebrafish glia are induced, by Fgf signalling, to form an elongated bipolar morphology that generates a bridge between the two sides of the resected spinal cord, over which regenerating axons actively regrow. Loss of Fgf function induced in the hsp70:dn-fgfr1 fish line, greatly inhibits glial cell proliferation and bipolar bridging across the lesion. Over-activation of Fgf signalling in sprouty 5-/- zebrafish or FGF8 injections into wildtype fish accelerates glial proliferation and promotes bridging morphology that subsequently supports axonal regeneration in vivo. In mouse, 2 weeks after SCI, Fgf2 injections increased radial glia marker expression, Pax6 and nestin, on proliferating cells. This results in GFAP expressing proliferation late in development.

POS-MON-018

PROLIFERATION IN THE MOUSE SYMPATHETIC GANGLION AND ITS REGULATION BY RET

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Purpose: Cell proliferation ceases with cell differentiation in the mouse CNS. We measured cell cycle dynamics during prenatal development of the mouse sympathetic stellate ganglion, where neuroblasts continue to proliferate following differentiation. Methods: Wild type and RetTGM/TGM embryos were treated in utero with the S-phase markers BrdU (2 h) and EdU (0.5 h) and were killed and processed for immunofluorescence for combinations of BrdU, EdU, tyrosine hydroxylase (TH), Sox10 and Kit. BrdU, EdU and Kit7 staining was used to identify S-phase, cell cycle length and growth fraction, while TH and Sox10 identified neuroblasts and both neural crest and glial cells respectively. A minimum of 3 embryos was examined at each age examined. Results: At E9.5, when neural crest-derived cells were migrating and coalescing into the sympathetic ganglion primordium, all cells were cycling, cell cycle length was only 10.6 hours and S-phase comprised over 65% of the cell cycle; these values are similar to those previously reported for embryonic stem cells. At E10.5, Sox10+ cells lengthened their cycle to 30 h and reduced the length of S-phase. As cells started to express TH on E10.5, they exited the cell cycle. At E11.5, when over 80% of cells in the ganglion were TH+ neuroblasts, all cells were again cycling. Loss of Ret increased neuroblast cell cycle length at E16.5 and decreased the number of neuroblasts at E18.5. Conclusion: Like other neurons, sympathetic neuron differentiation leads to exit from the cell cycle – sympathetic neurons are unusual in that they then re-enter the cell cycle before later permanently exiting. The GDNF family ligand co-receptor, Ret, regulates sympathetic neuroblast proliferation late in development.

POS-MON-019

THE POTENTIAL OF INDUCED PLURIPOTENT STEM CELLS FOR AUDITORY NEURON REPLACEMENT

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Purpose: Low numbers of auditory neurons (ANs) are thought to reduce the efficacy of the cochlear implant. Therefore research efforts in our laboratory are focused on replacing ANs using stem cells. The aim of this project is to examine the potential of induced pluripotent stem cells (iPSCs) for AN replacement. Methods: To characterise the potential of hESCs to differentiate into AN lineages, the iPSC lines (ES4CL1, ES4CL2) and the human embryonic stem cell (hESC) line (H9; control) were employed. An established in vitro assay was used to differentiate stem cells towards a sensory neural lineage. Immunocytochemistry was used to examine expression of seven key AN developmental markers at several time points of differentiation (19,21,24,28 and 35 days in vitro).

To investigate the potential of stem cells to innervate their peripheral targets (hair cells), the iPSC and hESC-derived neurospheres were co-cultured with cochlear explants (n=24) derived from early post-natal day 3-4 mice for 10 days in vitro. Results: Both the hESC and iPSC-derived neurospheres expressed the AN developmental markers; Pax7, Pax2, Brn3a, GATA3, Islet1, Neurofilament 160Kda, and βIII-tubulin over the time course examined (for each marker n>8). However the levels of marker expression were not identical between the three cell lines. Both cell types were able to extend their neural processes towards and along the rows of hair cells in cochlear explants. The innervation density of iPSC-derived neural processes towards hair cells was lower compared to hESCs. Conclusion: This data suggests that both iPSCs and hESCs have the potential to differentiate towards an AN lineage and innervate their peripheral targets but with variable efficiencies.

POS-MON-020

PERTURBATIONS IN CORTICAL DEVELOPMENT AND SEIZURE SUSCEPTIBILITY ARISING FROM PRENATAL EXPOSURE TO BENZODIAZEPINES IN MICE

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During brain development, many factors influence the assembly and final positioning of cortical neurons, and this process is essential for proper circuit formation and normal brain function. Among many important extrinsic factors that guide the maturation of the embryonic cortical neurons, the secreted neurotransmitter GABA has been proposed to influence their migratory behaviour, as well as their terminal differentiation. The full extent of the short and long-term changes on brain patterning and function caused by modulators of the GABA system is not known. We specifically asked if Diazepam, a commonly used benzodiazepine that modulates GABA receptor, alters embryonic neuronal patterning in vivo and whether it can lead to permanent effects on brain function. Fetal exposure to Diazepam did not change cell positioning within the E14.5 mouse cerebral cortex, but significantly altered neuron positioning in the E18.5 cortex, namely the distribution of mid-born (E14.5) interneurons, labelled by a GFP reporter in the Dlx5/6- Cre mouse line, as well as Tbr1 labelled early projection neurons. In adult mice, a significant change in cell positioning was evident in Cairetinin and Parvalbumin labelled interneurons in the upper layers of the cortex. Behaviourally, Diazepam altered seizure susceptibility in adult mice, as assessed by a proconvulsant challenge. Therefore, fetal exposure to Diazepam has consequences for neuron migration and postnatal behavioural changes.
POS-MON-021

IUGR CAUSES ALTERATIONS IN CTIP2- AND SOMATOSTATIN-EXPRESSING NEURONS IN THE CEREBRAL CORTEX: A POSSIBLE LINK TO AUTISM?

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Purpose: Intrauterine growth restriction (IUGR) can result in abnormal brain development, leading to neurodevelopmental sequelae in postnatal life. Recent epidemiological evidence suggests a link between prenatal compromise, including IUGR, and autism. Although the neuropathology of autism is unknown, imaging studies suggest that the neuropsychiatric symptoms are likely due to functional disturbances and cerebral disconnectivity. Here we investigate the impact of IUGR on specific neuronal subtypes in the cerebral cortex known to be affected in autism.

Methods: At 30 days of gestation (dg; term ~67dg), chronic placental insufficiency was induced by diathermic ablation of half of the branches of the uterine artery supplying the placenta to produce IUGR fetuses (n=7); controls (n=6) were from sham-operations. At 52dg sections of the cerebral hemispheres were immunostained to identify subcortical projection neurons (Citp2), GABAergic interneurons (somatostatin and calretinin) and microglia (Iba-1); areal density was determined and expressed as cells per mm². Results: There was a reduction (p<0.001) in Citp2-immunoreactive (IR) neurons and somatostatin-IR interneurons in the cerebral cortex in IUGR fetuses compared to controls; the subpopulation of calretinin-IR interneurons were not affected (p>0.05). There was no difference between groups (p>0.05) in the density of Iba-1-IR microglia. Conclusion: IUGR results in a decrease in both subcortical projection neurons and somatostatin-IR interneurons in the cerebral cortex. A reduction in these neurons could influence connectivity and possibly lead to cortical dysfunction as shown in autism. Analysis of other neuronal populations and the long-term neurofunctional implications of these findings in IUGR, need further investigation.

POS-MON-022

NFIX REGULATES ADULT NEUROGENESIS WITHIN THE SVZ AND RMS

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Neurogenesis within the adult brain occurs in two primary areas, the subventricular zone (SVZ) lining the walls of the lateral ventricles, and the subgranular zone of the hippocampal dentate gyrus. Within the SVZ, neural progenitor cells give rise to neuroblasts that migrate through the rostral migratory stream (RMS) into the olfactory bulb (OB), where they differentiate into interneurons. However, our understanding of the molecular regulation of these fundamental events remains limited. Here we demonstrate that the transcription factor Nuclear factor one X (NFX1) plays a key role in these processes. Nfx1 is expressed in the progenitor cells within the SVZ and RMS, and is also expressed within the OB. In Nfx1−/− mice there is an accumulation of cells within the SVZ. Moreover, Nfx1−/− mice display abnormalities in RMS structure, and in the migration of neuroblasts to the OB, which is ultimately smaller.

POS-MON-023

NEOGENIN REGULATES ADHERENS JUNCTION ASSEMBLY IN DEVELOPING CORTEX

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Purpose: With the onset of neurogenesis in the developing mouse neocortex, cortical radial progenitor cells undergo both symmetric and asymmetric division. Symmetric division results in the maintenance of the progenitor cell niche whereas asymmetric division gives rise to intermediate progenitors and neurons. One of the factors governing cell fate decisions of radial progenitors is apico-basal polarity, which is established by adherens junctions (AJ). It has previously been found that knockdown of Neoegenin results in the loss of neuroepithelial cell polarity in the zebrafish neural tube. Neogenin, an axon guidance receptor, is expressed in the radial progenitors of the neocortex. Thus, the purpose of this study was to determine the effect of loss of Neogenin in the apico-basal polarity of radial progenitors in the mouse neocortex.

Methods: The telencephalons of C57BL/6 embryonic (E) 12 embryos were transfected with Neoegenin shRNA or control scrambled shRNA and GFP constructs in vivo by in utero electroporation. The embryos were harvested at E14 and immunohistochemical staining was performed on sections. Results: Morphological analysis showed that loss of Neogenin resulted in loss of AJ integrity (visualised using ZO-1 marker) in the ventricular zone of the E14 neocortex in Neogenin shRNA knockdown (n=14) compared to scrambled shRNA controls (n=14). Furthermore, nuclear length-width ratio analysis of radial progenitors showed that nuclei in the Neoegenin shRNA knockdown neocortex were significantly more rounded compared to the scrambled shRNA controls, indicating that depletions of Neoegenin resulted in loss of apico-basal polarity (p<0.0015, Student t-test). Conclusion: Neoegenin is essential for the establishment of AJs and the maintenance of apico-basal polarity in radial progenitors of the developing neocortex.

POS-MON-024

SEMAPHORIN3A IS REQUIRED FOR THE AREAL PATTERNING OF THE VISUAL CORTEX

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Purpose: Throughout evolution the mosaic of visual cortical areas have expanded with the primates comprising many more areas than the rodent. These functionally unique areas are characterised by a specific cytoarchitecture and gene expression profile. However, the molecular regulators responsible for their establishment remain unknown. In the present study, we investigate the role of the guidance molecule Semaphorin3A (Sema3A) in establishing areal borders of known visual cortical areas in the mouse and marmoset visual cortex through segregation of migrating neurons to appropriate areas.

Methods: The expression profile of Sema3A and its receptor Neuropilin1 (Npn1) in the marmoset visual cortex (n=10) at 6 developmental stages were analysed by in situ hybridization and immunolabelling. We also performed volumetric analysis of the Sema3A KO mouse cortex (n=6) and in vitro migration assays on marmoset neuronal precursor cells.

Result: We demonstrate that Sema3A is uniformly distributed across the marmoset cortical plate at embryonic day (ED) 90, is restricted to layers 2 and 5 at ED110 and adopts an area-specific profile at birth, when Sema3A becomes heterogenously expressed across the marmoset visual cortex. For example, Sema3A is absent from the primary visual area V1 and strongly expressed in adjacent area V2. As Sema3A is attractive for Npn1+ cortical neurons, they preferentially populate Sema3A-enriched cortical areas, leading to selective migration into V2 and not in V1. In the mouse, loss of Sema3A leads to defective pyramidal neurons and reduction of the visual cortex volume compensated by an expansion of the somatotenary barrel field S1.

Conclusion: We provide evidence that the Sema3A/Npn1 pathway is involved visual cortex patterning by sorting/directing neuronal subpopulations. Furthermore, the pathway is conserved throughout evolution with primate-specific modifications.
POSTERS Monday

POS-MON-025

CONTROL OF PTEN SUBCELLULAR LOCALISATION BY UBIQUITINATION REGULATES CELL CYCLE PROGRESSION

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Purpose: PTEN is a major regulator of cell division and growth in the brain with functions including the negative regulation of the Akt/mTOR pathway. Neurological features of inherited PTEN loss include macrocephaly, seizures, and mental retardation. Recent controversial findings suggest that nuclear PTEN has important functions that are independent from the regulation of Akt at the cell surface. To investigate the role of nuclear PTEN we have modulated the levels of Ndfip1, a key protein controlling the ubiquitin trafficking of PTEN to the nucleus. Methods: PTEN nuclear localisation was inhibited through the loss of Ndfip1 in cell lines through both genetic deletion and RNAi. Cell proliferation was measured using MTT assays and cell cycle analysis performed using flow cytometry. Genetic mouse models for loss (knockout) or increase (transgenic expression) in Ndfip1 levels were investigated for PTEN localisation during brain development. Results: Loss of nuclear PTEN resulted in a rapid proliferation of cells, despite the cytoplasmic levels of PTEN remaining constant. Significant increases in the levels of the oncogenic proteins cyclin D1 and Pdk1 were observed in these cells along with a shortening of the G2/M phase of the cell cycle. In vivo we observed that neuronal progenitors of the SVZ had a shortened G2/M phase during development in Ndfip1 knockout mice, however, OxR2 expression in brain size or neuronal layering was observed. In contrast, brains from transgenic animals over-expressing Ndfip1 were found to be significantly smaller with no increase in cell death observed. Conclusion: We find that nuclear PTEN has important functions for maintaining cell cycle that are independent from its well known cytoplasmic role in regulating Akt. Ubiquitination of PTEN is mediated by Ndfip1 and is required for nuclear translocation during the cell cycle.

POS-MON-027

EFFECTS OF POSTNATAL NICOTINE EXPOSURE ON OREXIN RECEPTORS 1 AND 2 IN THE DEVELOPING PIGLET HYPOTHALAMUS

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Purpose: Infants exposed to passive cigarette smoke have been shown to have perturbed orexin (OXA) responses. Orexin affects arousal, and regulates appetite and sleep via its receptors 1 and 2 (OxR1 and OxR2). In piglets exposed to post-natal nicotine at levels equivalent to those observed in infants exposed to cigarette smoke, we investigated the effects of nicotine on OxR1 and OxR2 protein expression in the hypothalamus. Methods: Two piglet groups were studied: control (n=14) and nicotine (n=14). Seven nuclei/areas of the hypothalamus were studied using immunohistochemistry: the paraventricular nucleus (PVN), dorsal medial nucleus (DMN), arcuate nucleus (ARC), ventral medial nucleus (VMN), perifornical area (PFA), lateral hypothalamic area (LHA), lateral tubular nucleus (LTu) and the tubular mammillary nucleus (TMN). Results: Compared to controls, OxR1 and OxR2 expression were increased by nicotine exposure in a region-dependent manner. OxR1 expression was increased in the DMN (P<0.001) and the LTu (P=0.04), OxR2 expression was increased in the DMN (P<0.001), VMH (P=0.01), and the TMN (P=0.03). After nicotine exposure, females had greater expression than males for both OxR1&2 in the DMN. Conclusions: Nicotine exposure increases orexin receptor expression in regions of the hypothalamus known to express nicotinic acetylcholine receptors (nAChR) (DMN & VMH) (Block and Billar, 2000, Hill et al., 1993). As these regions participate in the regulation of sleep and arousal, this could contribute to the dysregulation of arousal seen in infants exposed to cigarette smoke.

POS-MON-026

APP STIMULATES NEURAL STEM/PROGENITOR CELL PROLIFERATION BY INCREASING CYSTATIN C SECRETION

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The amyloid precursor protein has been well studied for its role in Alzheimer’s disease. However, little is known about its normal function. In this study, we examined whether APP plays a role in neural stem or progenitor cell proliferation. Neural stem / progenitor cells (NSPCs) were cultured as neurospheres. We found that there was an increase in the proliferation of NSPCs derived from APP-overexpressing Tg2576 transgenic mice (P<0.05, n=7). However, NSPCs obtained from APP knockout mice (APP KO mice) had reduced proliferation rates (P<0.01, n=4). To examine if a secreted factor was responsible for the increased proliferation, the conditioned medium (CM) was collected from Tg2576, APP KO and wide-type neurosphere cultures and added into the neurosphere-derived cell cultures. CM from Tg2576 cultures stimulated neurosphere proliferation (P<0.05, n=5) compared to wild-type CM whereas CM from APP KO cultures had little effect on proliferation of neurosphere. To identify the secreted molecules in the CM of Tg2576 cultures which can promote proliferation, the CM was analyzed for its effect on proliferation by immunoblotting and immunodepletion. Immunodepletion of APP from CM of Tg2576 did not reduce proliferation (P>0.05, n=4). Furthermore, recombinant APP did not stimulate proliferation. As cystatin C has been reported to stimulate neural stem cell proliferation, we examined the possibility that the secreted molecule may be cystatin C. Levels of cystatin C were higher in the CM of Tg2576 cells than in the CM of WT cells (P<0.05, n=9). Cystatin C was lower in the CM obtained from APP KO cultures (P<0.05, n=3). Immunodepletion of cystatin C from CM of Tg2576 culture decreased the effect on cell proliferation (P<0.05, n=4). The results demonstrate that APP stimulates NSPC proliferation, and that this effect is mediated via an increase in cystatin C secretion.

POS-MON-028

DEVELOPMENTAL EXPRESSION OF NURR1 / NR4A2A IN A NOVEL TRANSGENIC ZEBRAFISH LINE

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During neural development, factors instruct progenitors to generate diverse subtypes, each carrying out distinct functions. The Nurr1/Nr4a2a orphan receptor has been shown to be crucial for the development of dopaminergic neurons in various central nervous systems (CNS) regions including the substantia nigra and retina, and has been linked to familial Parkinson’s disease. Nr4a2a is, however, also expressed in non-dopaminergic neuron subtypes. Purpose: Generate a transgenic zebrafish line Tg(nr4a2a:GFP). Use the rapid ex vivo development of zebrafish to characterise the spatio-temporal expression of this dopamine-dependent zebrafish, focusing on the well characterised retinal subtypes and building on our work on neural lineages within the retina. Methods: Clone the nr4a2a promoter to drive GFP using the Gateway recombination system. Generate (n = 400) and characterise founders. Compare transgene with endogenous RNA expression (in situ hybridisation) and describe Nr4a2a:GFP expression within different CNS areas at different ages (n = 50 embryos / founder). Characterise different GFP neuron subtypes immunohistochemically. Results: Injections of nr4a2a:GFP resulted in mosaic expression. Developmental expression patterns were evaluated in 6 founders. In offspring from one founder, we compared GFP vs endogenous RNA expression. Tyrosine hydroxylase staining confirmed Nr4a2a:GFP expression in dopaminergic neurons. Conclusions: We successfully generated a novel zebrafish line, in which Nr4a2a:GFP expression can be followed developmentally in fixed and in vivo studies. This line is useful for assessing consequences of functional manipulation of factors including Nr4a2a, to provide real-time insight of alternative lines of progenitors. Characterising alternate fates might identify a new source of cells with dopaminergic potential that can be targeted by therapeutic approaches.
CELL INTRINSIC AND EXTRINSIC FACTORS ENHANCE NEURAL CIRCUIT RECONSTRUCTION FOLLOWING TRANSPLANTATION IN PARKINSONIAN MICE

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Purpose: Cell transplantation for Parkinson’s disease has predominantly focused on ectopic transplantation of fetal dopamine (DA) neurons into the striatum as a means to restore neurotransmission, rather than homotopic grafts into the site of cell loss, which would require extensive axonal growth. However, ectopic grafts fail to restore important aspects of DA circuitry necessary for controlled basal ganglia output, and this may underlie the suboptimal and variable functional outcomes in patients. We recently showed that DA neurons in homotopic allografts of embryonic ventral mesencephalon (VM) can send long axonal projections along the nigro-striatal pathway in order to innervate forebrain targets, however the extent of striatal reinnervation remains substantially less than can be achieved with ectopic placement directly into the striatal target.

Methods: In 109 transplanted animals, we examined the possible benefits of using younger VM donor tissue, and striatal over-expression of glial-cell derived neurotrophic factor (GDNF) in order to improve the degree of striatal innervation from homotopic grafts. Results: Younger donor tissue, collected on embryonic (E) day 14, generated 4-fold larger grafts with greater neuronal and striatal targeting, compared to grafts of conventional E12 donor VM. GDNF over-expression also significantly increased DA axonal growth and striatal innervation. Furthermore, a notable increase in the number and proportion of A9 DA neurons, essential for functional recovery, was observed in younger donor grafts treated with GDNF. Behavioural testing confirmed functional integration of younger donor tissue and demonstrated that improved motor function could be attributed to both local midbrain and striatal innervation.

Conclusion: These findings suggest there is significant scope for further development of intra-nigral grafting as a restorative approach for PD.

ROLE OF THE FRAGILE X MENTAL RETARDATION PROTEIN DURING BRAIN DEVELOPMENT

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Introduction: Fragile X Mental Retardation Protein (FMRP) is an RNA binding protein highly expressed in brain. It is involved in RNA metabolism in neurons and its absence causes Fragile X Syndrome (FXS), the most common form of inherited mental retardation. FXS is characterized by dendritic spine dysmorphogenesis. FMRP regulates a subset of mRNAs involved in spine structure and function. Purpose: Due to shared mechanisms based on cytoskeleton remodeling that lead to spine formation, maturation and pruning, we investigated the possible role of FMRP in cell migration. Methods: Wild Type (WT) and FMRP Knock-Out (KO) mice embryos were electroporated in-utero with a GFP expressing construct and neuronal cell migration phenotype was analysed in cortical slices several days later. Immunohistochemistry and molecular biology techniques were used to verify the observed phenotype. Results: We report that absence of FMRP results in accumulation of electroporated precursor cells in the lower part of the developing cortex. This phenotype was observed in 5 different experiments and is statistically significant. In addition, RNA-IP and quantitative PCR show that FMRP binds and possibly regulates the expression of multiple mRNAs involved in cell migration. Conclusions: Our findings suggest that FMRP plays a key role in neuronal migration during brain development. Alteration of this process may set the conditions for the development of the defects observed in FXS and may lead to the identification of new pharmacological targets for FXS treatments.

LRP-1 AND LRP-2 LIGANDS IN A NOVEL MECHANISM OF NEURONAL GROWTH CONE GUIDANCE

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Axon guidance is essential in the developing nervous system as well as in regeneration. Given the emerging role of low density lipoprotein receptor-related protein 1 (LRP-1) and LRP-2 receptors in neuronal growth and injury, we asked whether LRP-1 or LRP-2 were able to mediate neuronal guidance. We used chemotaxis assays, immunocytochemistry to establish LRP-1 and LRP-2 expression on the leading edge and filopodia of sensory growth cones. Embryonic (E16-18) rat sensory neurons were used in the in vitro growth cone turning assay to test a range of LRP ligands for chemotactic effects; including metallothionein II (MTII), apolipoproteinE3, tissue plasminogen activator, vitamin D and transthyretin. Three LRP ligands were chemotactic: Growth cones grew towards a microgradient of MTII (+9.8±1.7, p<0.0001, control -1.6±1.1), and away from a d2M (-11.9±3.4, P<0.01) and MTII1, a protein closely related to MTII (+13.8±1.9, p<0.0001). We examined the mechanism of the novel chemoattractant guidance cue, MTII. Growth cone turning towards MTII was abolished by pan-LRP-receptor inhibitor, RAP (0.6±1.2), and siRNA knockdown of LRP-1 (3.5±1.9) or LRP-2 (3.6±2.5). These data demonstrate that both LRP-1 and LRP-2 are necessary for MTII-induced chemotactic signal transduction. Growth cone turning towards MTII was reversed in response to inhibition of TrkA with K252a, showing TrkA involvement downstream of LRP-1 and LRP-2 activation. MTII chemoattraction was dependent on extracellular calcium; removal of extracellular calcium ions abrogated the attractive response. Furthermore, pharmacological inhibition of calcium/calmodulin-dependent kinase II suggests that LRP-1 and LRP-2 signal via established downstream effectors. LRP-mediated MTII chemotaxis represents a novel, non-classical axon guidance system that may be exploited in repairing the injured nervous system.

LAMINATION OF THE COMPLEX NONHUMAN PRIMATE VISUAL CORTEX

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Purpose: Corticogenesis is a complex process in which progenitor cells in the ventricular zone (VZ) and the subventricular zone (SVZ) give rise to the six mature layers of the neocortex in a spatiotemporal pattern during gestation. To further understand this process in humans, experiments in nonhuman primates will help identify both the conserved and unique features of this lamination. Our aim is to identify cells that were generated at different stages of gestation and their areal and laminar location in the visual cortex, which occupies ~50% of the neocortex. This will help us determine if there is homogenous or heterogenous development across the different areas of the visual cortex.

Methods: Pregnant marmoset monkeys (Callithrix jacchus, n=2) were pulsed with the thymidine analogues 5-bromo-2′-deoxyuridine (BrdU; 57.5mg/kg) and 5-chloro-2′- deoxyuridine (CldU; 42.75mg/kg) twice a day for three consecutive days. One animal received IdU at E60 and CldU at E80, while the other received IdU at E70 and CldU at E90. Offspring were subsequently humanely euthanized on postnatal day 1. Immunohistochemical analysis of these brain sections (40μm) with anti-CldU and anti-IdU antibodies were performed to identify cells expressing thymidine analogues.

Conclusion: By tracking the proliferative cells born at different stages of embryonic development using thymidine analogues, we can understand the spatiotemporal patterning of cortical lamination.

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DIRECT GENERATION OF NEURAL PRECURSORS FROM ADULT HUMAN FIBROBLASTS USING VIRAL GENE DELIVERY

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Purpose: Somatic cell reprogramming is an innovative field with considerable potential to enhance our understanding and treatment of a wide range of neurological disorders. We have recently demonstrated that adult human dermal fibroblasts (aHDFs) can be directly converted to neural precursors, by-passing an intermediate pluripotent embryonic stem cell stage. We have shown that induced neural precursors (iNPs) express a wide range of neural precursor and pro-neural genes and can be differentiated into mature, functional neurons expressing GAD65/67, glycine and tyrosine hydroxylase. For future clinical application this was achieved by over-expression of the transcription factors Sox2 and Pax6 using a viral transduction method. This study, however, aimed to develop a more efficient non-clonal protocol using lentiviral gene delivery for direct generation of neural precursors from aHDFs.

Methods: We constructed lentiviral vectors expressing Sox2-zsGreen (LV-Sox2-zsGreen) or Pax6-tdTomato (LV-Pax6-tdTomato) and optimized lentiviral transduction of aHDFs. FACS was used to determine the efficiency of LV transduction and quantitative PCR was used to identify the expression of pro-neural genes in resulting iNP colonies.

Results: Viral transduction efficiency (~40%) was higher than for non-viral transfection (~10%) (n=3 wells for 2 independent lines). Further, ~9.3% of aHDFs exhibited co-expression of Sox2 and Pax6. We observed an increase in the rate of iNP colony formation (~14-20 days) compared to non-viral transfection (~65-85 days). Quantitative PCR analysis confirmed lentiviral-induced neural precursors express a wide range of neural precursor and pro-neural genes indicating efficient reprogramming to a neural precursor-like state.

Conclusion: Our results suggest viral induction of neural precursor cells from aHDFs is more efficient and better suited for research purposes than non-viral methods.

POSTERS  Monday

TRANSMEMBRANE DOMAIN NRG1 MUTATION DOES NOT REPRESENT NRG1 ‘KNOCKOUT’

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Purpose The Nrg1 transmembrane domain mutant (Nrg1 TM) mouse has been used to investigate the role of NRG1 in schizophrenia-like behavioural phenotypes. However, the molecular alterations in this mouse and their resemblance to alterations in the brain in schizophrenia remain unclear. Early studies implied that the Nrg1 TM mouse was a hemizygous ‘knockout’ model of reduced Nrg1 function (i.e. binding to ErbB receptors), however, molecular evidence for Nrg1 reduction in the brain of the Nrg1 TM mouse is lacking. Methods Using probes for the cysteine-rich, bioactive epidermal growth factor (EGF)-like, transmembrane and intracellular domains of Nrg1 and for the NMDA receptor subunits NR1, NR2A, NR2B, NR2C and NR3D, mRNA was measured using quantitative RT-PCR in frontal cortex homogenates from male and female Nrg1 TM and wild type-like (WT) mice at postnatal day 7, 10, 14, 21, 35, 49 and 161. Results Nrg1 TM mice have ~40% reduced expression (mean ± SEM) of the transmembrane domain of Nrg1 (Nrg1 TM: 109.8 ± 5.1; WT 63.4 ± 2.7), but unchanged expression of the EGF-like domain (Nrg1 TM: 71.4 ± 3.5; WT: 74.4 ± 2.8) and intracellular domain (Nrg1 TM: 88.8 ± 3.5; WT: 85.9 ± 6.6). NMDAR subunit expression follows defined developmental trajectories but is not impacted by the Nrg1 mutation. Conclusion ErbB receptor binding capacity does not appear ‘knocked out’ in the Nrg1 TM mouse. Transcription downstream from the transmembrane domain may continue via alternative splicing or promoter usage.

DIFFERENTIAL PRESYNAPTIC GABA-A AND GABA-B MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION IN DORSAL RAPHE NUCLEUS

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The dorsal raphe nucleus (DRN) projects serotonergic axons throughout the brain to influence emotional states. A complex network of excitatory and inhibitory axons regulates DRN neurons. In the present study, recordings were made from putative serotonergic neurons within the DRN based upon their size and action potential characteristics. Miniature excitatory and inhibitory postsynaptic currents (IPSCs and EPSCs) were readily distinguished as outward and inward synaptic currents in DRN neurons, voltage clamped at -55 mV. The GABA_A agonist, baclofen (10 µM), produced a gradual reduction in the frequency of miniature EPSCs in all DRN neurons tested, but had no effect on the amplitude of miniature EPSCs. On average, the rate and amplitude of miniature EPSCs was 32 ± 5% and 99 ± 4% in the presence of baclofen compared to pre-application levels (p < 0.0001 & p = 0.8, n = 7). In contrast, the GABA_A agonist, muscimol (2 µM), produced a rapid increase in the frequency of miniature EPSCs in all DRN neurons tested. Muscimol had no effect on the amplitude of miniature EPSCs in 7/10 neurons. On average, the rate and amplitude of miniature EPSCs was 175 ± 18% and 112 ± 8% in the presence of muscimol compared to pre-application levels (p = 0.003 & p = 0.15, n = 10). These findings indicate that GABA_A has the potential to both inhibit and enhance glutamate release in the DRN via presynaptic GABA_A receptors, respectively. This may be an important mechanism for the regulation of physiological and psychological states.
POS-MON-037

GABA AND GLYCINE CONTENT OF VESICLES AT MIXED INHIBITORY SYNAPSES

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Aims: Individual presynaptic vesicles from mixed inhibitory neurons in the mammalian spinal cord and brain stem can co-release glycine and γ-aminobutyric acid (GABA) because these two transmitters are accumulated into vesicles by the same vesicular transporter named VIAAT (or VGAT). Thus, the inhibitory transmitter content of synaptic vesicles is thought to be determined solely by the ratio of glycine and GABA available in the cytoplasm, and vesicles originating from the same presynaptic terminal should release a homogenous ratio of transmitter.

Methods & Results: We test this hypothesis using electrophysiological techniques first in a model sniffer-cell giant-synapse and then in cultured spinal cord neurons derived from eGFP-GlyT2 mice. In our model synapse, where VIAAT expressing synaptic-like vesicles had access from a single synapse was heterogeneous: mixed events along with glycine and/or GABA events were detected (n > 18). We confirmed that this heterogeneity originated from the presynaptic terminal, as GABA and glycine receptors were evenly expressed on the membrane of the model post-synaptic cell (n = 4). Next we grew low density primary cultures of spinal cord neurons and identified varicosities that were 1.05 ± 0.3 × 1.50 ± 0.3 μm and could be labelled with FM4-64 dye (76%), corresponding to inhibitory presynaptic boutons. Then we sampled the single bouton vesicle content (n = 16) of glycinergic and mixed inhibitory boutons using a combination of loose-patch and whole-cell patch-clamp electrophysiology.

Conclusions: In the brainstem and spinal cord the inhibitory signal may be finely tuned at the level of the presynapse.

POS-MON-039

OPTIMAL REPETITION RATE AND BREAK DURATION FOR APPLICATION OF ANODAL-TRANSCRANIAL DIRECT CURRENT STIMULATION

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Purpose: To explore the effects of three different repetitions and two break durations of a-DCS-induced corticospinal excitability (CSE) changes over FDI muscle primary motor cortex. Method: 12 healthy right-handed individuals participated in a blind, single-blind, crossover study with washout period. Five different conditions were compared: 1) 10 min a-DCS (10-0-0), 2) 10 min a-DCS-5 min break-10 min a-DCS (10-5-10), 3) 10 min a-DCS-25 min break-10 min a-DCS (10-25-10), 4) 10 min a-DCS-5 min break-10 min a-DCS-5 min break-10 min a-DCS (10-5-10-5-10) and 5) 10 min a-DCS-25 min break-10 min a-DCS-25 min break-10 min a-DCS (10-25-10-25-10). A-DCS was applied by a plasticity-inducing protocol.

Results: Baseline values were identical for all conditions (P=0.05). A two-way ANOVA showed significant main effects of condition (P=0.003) and time (P=0.005). Post-hoc comparisons showed significant differences between 10-0-0 and both 10-25-10 (P=0.001), 10-5-10-5-10 (P=0.001) conditions. Also, there were significant differences between 10-5-10 and both 10-25-10 (P=0.001) and 10-25-10-25 (P=0.004) conditions. Compared to baseline value, CSE was significantly increased in all conditions after the completion of the tests (P=0.000). This increase remained significant up to two hours for the conditions with two and three repetitions. Conclusion: The conditions with 25 min of break induced maximum changes. However, there were no significant differences between the conditions with two and three repetitions. Overall, the findings indicate that the duration of the break is the key factor for the size of CSE changes.

POS-MON-040

THE RELATIONS BETWEEN MEMBRANE OSCILLATIONS AND SPIKE THRESHOLD

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Purpose: Information processing depends not only on the electrophysiological properties of neurons but also on their dynamical properties. As many cortical, thalamic and hippocampal neurons exhibit oscillatory potentials, we examined their internal properties in response to membrane oscillations. Recent studies have shown that the resonance frequency of the soma and single dendrites are voltage-dependent and change following synaptic plasticity. However, little is known about their suprathreshold membrane oscillations properties. Rheobase is the minimal current in indefinite time required to elicit a spike, and is a valid measure for cell excitability. As resonating neurons respond to inputs in a preferred frequency, and are thus limited to currents with short duration, we measured the rheobase to a sinusoidal current (sin-rheobase). We than tested the relationship between alterations in oscillation frequency and the spike threshold. Method: Fixed amplitude sinusoidal current with increasing frequency (Chirp stimulation of 0-100, 0-200 or 0-300 Hz for 10 sec) was applied through the recording electrode. Small increments in the current amplitude were applied until a spike was elicited. The sin-rheobase was determined as the minimal current amplitude in which a spike was initiated in a certain frequency. Results: Both excitatory (n=4) and inhibitory (n=3) neurons showed line correlation between the sin-rheobase and the oscillating frequency, as spike threshold increased in higher oscillating frequencies. The Amplitude-Frequency curve changed between cells, and was correlated to the membrane time constant. Conclusion: These results suggest that neuronal excitability changes following alterations in oscillation frequencies. Moreover, as different neurons had different sin-rheobase-frequency signatures, it might allow the discrimination between subclasses of neurons.
EXERCISE TRAINING ALTERS SYNAPTIC PROPERTIES OF SPINAL NEURONS AFTER INCOMPLETE SPINAL CORD INJURY IN ADULT MICE

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Following spinal cord injury (SCI), anatomical changes such as axonal sprouting occur within weeks in the vicinity of the injury. Exercise training enhances axon sprouting, however, the mechanisms that mediate exercised-induced plasticity are unknown. Purpose: To examine the influence of 3 and 6 wks of exercise training on intrinsic and synaptic properties of neurons in the vicinity of an SCI. Methods: Adult mice (C57Bl/6; n=36) received a spinal hemisection (T9-10) and, after 1 wk of recovery, were assigned to untrained and trained (10 min treadmill exercise, 5 wk for 3 or 6 wks) groups. Mice were sacrificed (100 mg/kg, i.p. ketamine, decapitation) and horizontal spinal cord slices were prepared for patch clamp recording. Results: Most intrinsic properties were similar in untrained and trained groups at both time points (3 wk untrained = 65 neurons, trained = 66, 6 wk = 61 and 65). Input resistance decreased in the 6 wk trained mice (856.0 ± 55.3 vs. 811.2 ± 78.1 MΩ). The proportions of tonic firing, initial bursting, single spiking and delayed firing neurons were similar in untrained and trained groups at both time points. The amplitude of spontaneous EPSCs was higher in trained animals at 3 and 6 wks (22.3 ± 0.9 vs. 18.7 ± 0.8 pA and 24.1 ± 1.4 vs. 16.7 ± 0.6 pA). EPSC amplitude, evoked by dorsal column stimulation, was enhanced in 3-wk trained animals (78.9 ± 17.5 vs. 42.2 ± 6.8 pA). Conclusion: Exercise after SCI selectively alters synaptic properties of spinal neurons in adult mice.

OPTGENETIC ANALYSIS OF EXCITATORY CIRCUITS IN THE PIRIFORM CORTEX

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Targeted expression of light-activated proteins provides a powerful method for the analysis of neuronal circuits. We have generated a transgenic mouse line (48L) in which selective expression of a tetra-cycline-dependent transactivator (ITA-mCitrine) allows us to drive expression of channelrhodospin-2 (ChR2) in a subtype of glutamatergic neurons in the primary olfactory (piriform) cortex. Purpose: To confirm the identity of ITA-expressing neurons in 48L mice, establish ChR2 expression, and use this system to map some of the excitatory circuits in the piriform cortex. Methods: Adeno-associated virus containing a ChR2 construct (TRE-ChR2-mCherry) was stereotaxically injected into 48L mice (P1-2). After >20 days, slices of the piriform cortex were prepared and whole-cell recordings made from histologically-verified layer 2 neurons at 32 ± 1°C. Light-driven currents were recorded in the presence of 100μM picrotoxin, 100μM 4-AP and 0.5μM tetrodotoxin. Results: All neurons expressing ITA-mCitrine showed electrophysiological properties typical of semilunar (SL) cells (n=26). In virus-injected animals (n=8), ChR2 expression was restricted to mCitrine-positive (i.e. SL) neurons. Photostimulation reliably produced large inward currents (155 ± 54 pA, n=23) and no response in ChR2-negative SL neurons (n=29). In contrast, photostimulation produced inward currents in 19% of layer 2 superficial pyramidal (SP) neurons (n=36 tested). Conclusions: The 48L mouse enables selective optogenetic manipulations of SL cells in the piriform cortex. Our experiments indicate that SL cells excite SP cells, but not other SL cells, confirming less-direct evidence from our previous work.

ZOLPIDEM INTERACTIONS WITH GABAa RECEPTORS IN HUMAN BRAIN SHOW MARKED REGIONAL VARIATIONS IN OCCIPITAL CORTEX

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Purpose: To underpin in vivo PET studies of GABAa receptors we explored the relationships of subunit-selective and -nonselective modulators, Zolpidem and GABA, on the binding of a range of informative radiotracers. Flumazenil binds to GABAa receptors containing a α subunit to portray overall receptor density; Ro15-4513 is selective for α5-containing receptors. Alcoho show reduced binding of [14C]flumazenil and [14C]Ro15-4513 to alcoholics and patients with drug addiction and bipolar disorder. In this study, we determined the pharmacological and quantitative characteristics of GABAa receptors in the occipital lobe. Methods: To determine the pharmacological and quantitative characteristics of GABAa receptors in the occipital lobe. Results: All radiotracers showed high affinity for GABAa receptors in the occipital lobe. The highest density was in occipital cortex. There were no significant differences between alcoholic cases and controls on these parameters. Conclusion: These data suggest there are marked variations in α-subunit composition in human brain, which may confound analysis of PET data, notably in occipital cortex.
POS-MON-045

PROPERTIES OF CHOLINERGIC AND NON-CHOLINERGIC SUBMUCOSAL NEURONS ALONG THE MOUSE COLON

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Purpose: The mouse has emerged as the research model for several enteric pathologies, however much is still unknown about murine submucosal neurons. Thus, we examined the properties of cholinergic (contains choline acetyltransferase, ChAT) and non-cholinergic submucosal neurons in the mouse colon. Methods: Methods: ChAT-Rosa (yellow fluorescent protein, YFP) mice (mixed background, progeny of ChAT-Cre X Rosa (YFP) reporter mice) express YFP in every neuron that has ever contained ChAT. Submucosal preparations were obtained from proximal, middle and distal colonic regions of ChAT-YFP and control C57Bl6 mice. The proportion of submucosal neurons (indicated by pan-neuronal marker Hu) that expressed YFP, CHAT and/or vasoactive intestinal peptide (VIP) was examined immunohistochometrically. The electrophysiology and morphology of submucosal neurons in the distal colon were studied using intracellular microelectrodes containing biocytin. Results: The majority of submucosal neurons lacked YFP but expressed VIP. The number of submucosal neurons/ganglion decreased while the proportion of VIP+ neurons increased distally along the colon. In the distal colon, submucosal neurons had one (52/55) or two axons (3/55), and did not supply varicosities to neighbouring ganglia. Somatic action potentials were insensitive to tetrodotoxin (1 μM, Na+ channel blocker, n = 5). Prolonged after-hyperpolarizing potentials were never observed. Most neurons exhibited fast excitatory postsynaptic potentials (fEPSPs, 77/78), that were abolished by hexamethonium (200 μM, nicotinic receptor blocker, n = 9). Veratridine (1 μM, Na+ channel blocker) induced an increase in fEPSPs (n = 8) that were blocked by hexamethonium (n = 2). Conclusion: There is a gradient for cholinergic neurons along the colon. There is no evidence for typical sensory neurons or non-cholinergic fast excitatory neurotransmitters in the submucous plexus of the mouse distal colon.

POS-MON-046

PHASIC AND TONIC FORMS OF GLYCINERGIC INHIBITION REGULATE PARVALBUMIN POSITIVE DORSAL HORN NEURON EXCITABILITY

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Purpose: The spinal dorsal horn is a critical site for processing sensory information related to nociception, touch, temperature and itch. Importantly, segregation of these modalities is essential for contextually relevant sensory experience and when this fails pathological sensations such as allodynia emerge. We recently described a population of inhibitory parvalbumin-positive interneurons (PVINs) with connectivity and functional properties that enable them to prevent touch related information from escaping nociceptive circuits. Unexpectedly, PVINs received only weak excitatory input, which is surprising as the output of neural circuits is governed by balanced excitatory and inhibitory synaptic inputs. Here we examine inhibitory drive to PVINs. Methods: Inhibition was assessed using targeted patch-clamp recordings in transgenic mice, expressing enhanced green fluorescent protein (eGFP) in PVINs. Mice (2-3 months, n = 5) were anaesthetized (ketamine 100 mg/kg i.p.), decapitated, and transverse slices were prepared from lumbar spinal cord. Results: Phasic and tonic forms of inhibition were detected in all PVIN recordings. Miniature inhibitory post synaptic currents (mIPSCs) had large amplitudes (124 ± 23 pA, n = 9), fast rise (0.78 ± 0.09 ms) and decay times (6.12 ± 0.54 Hz), and occurred at relatively high frequency (1.31 ± 0.25 Hz). Bath application of bicuculline (10 μM) had little effect on mIPSC properties, whereas addition of strychnine (1 μM) completely abolished mIPSCs. This suggests phasic inhibition from glycineric inputs is dominant in PVINs. Additionally, strychnine application reduced the holding current in all PVIN recordings (104.69 ± 52.18 pA) indicating that tonic inhibition from glycineric sources also regulates activity in PVINs. Conclusion: Our data indicate glycineric inhibition regulates PVIN function and can alter the capacity of spinal circuits to process and separate touch and nociceptive signals.

POS-MON-047

CHARACTERIZATION OF NEUREXIN-NEUROLIGIN/ LRRTM USING IN VITRO CELL BINDING ASSAYS

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Purpose: Vertebrate studies have shown Neurexin-Neuroligins/LRRTMs as binding partners in a trans-synaptic cell adhesion complex, implicated in human autism spectrum disorders and schizophrenia. The Neurexin-Neuroligin/LRRTM adhesion system of synapses is highly conserved across species highlighting that these proteins are critical for connectin the pre- and post-synaptic neurons, modulating neuronal circuitry in the brain and link the abnormalities in BDNF secretion to brain development and cognitive function. The results tell us that insect neuropeptides, BDNF is preferentially synthesized in, and secreted from glutamatergic excitatory neurons via exocytosis. Despite the importance of BDNF release in brain development and plasticity, the cellular and molecular mechanisms regulating BDNF secretion and their role in interneuron development are largely unknown. Intriguingly, we found that the activity-dependent BDNF secretion in excitatory neurons modulates GABAergic interneuron synapse formation on excitatory neurons. Furthermore, we identified the novel molecular mechanism of how the activity-dependent BDNF secretion in excitatory neurons regulates GABAergic interneuron synapse formation on excitatory neurons. Our findings provide fundamental insights into the cellular and molecular mechanisms that regulate the development of neural circuitry in the brain and link the abnormalities in BDNF secretion to cognitive disease.

POS-MON-048

THE ROLE OF BDNF EXOCYTOSIS IN GABAERGIC INTERNEURON SYNAPSE FORMATION

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Brain-derived neurotrophic factor (BDNF) is a small, secreted protein that plays a fundamental role in nervous system development and in regulating the strength of existing synapses throughout adult life. Imbalances in BDNF signaling impair several forms of neuronal plasticity and lead to a wide range of cognitive abnormalities. Unlike classical neurotransmitters, BDNF is preferentially synthesized in, and secreted from glutamatergic excitatory neurons via exocytosis. Despite the importance of BDNF release in brain development and plasticity, the cellular and molecular mechanisms regulating BDNF secretion and their role in interneuron development are largely unknown. Intriguingly, we found that the activity-dependent BDNF secretion in excitatory neurons modulates GABAergic interneuron synapse formation on excitatory neurons. Furthermore, we identified the novel molecular mechanism of how the activity-dependent BDNF secretion in excitatory neurons regulates GABAergic interneuron synapse formation on excitatory neurons. Our findings provide fundamental insights into the cellular and molecular mechanisms that regulate the development of neural circuitry in the brain and link the abnormalities in BDNF secretion to cognitive disease.
POS-MON-049
CANNABINOID RECEPTOR INTERACTING PROTEIN (CRIP1a) MODULATES CB1 RECEPTOR MEDIATED SIGNALLING

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Introduction: The primary cannabinoid receptors in the central nervous system (CNS) are CB1 receptors. Cannabinoid receptor interacting protein (CRIP1a) binds to and interacts with the C-terminal tail of the CB1 receptor (aa 418-472) and has been shown to suppress the tonic inhibition of voltage-gated Ca2+ channels induced by CB1 receptors in neurons (Niehaus et al., 2007). Activation of CB1 receptors activates G-proteins and therefore inhibits adenylate cyclase, CAMF formation and Ca2+ mobilisation. Furthermore it activates G-protein coupled inwardly rectifying potassium (GIRK) channels and causes phosphorylation of proteins in the MAP kinase pathway including ERK1/2. Therefore CRIP1a provides a new avenue for modulation of the endocannabinoid system in the CNS where it is known to have beneficial effects. Results: Western blot analysis of protein samples showed all three CRIP1a-siRNAs to induce a significant reduction in CRIP1a protein levels (p < 0.01). Furthermore, quantitative RT-PCR analysis showed a corresponding reduction in mRNA levels (p < 0.05) in two of the three siRNAs screened. Candidate downstream signalling pathways are currently being screened to investigate the effect of decreased CRIP1a on modulation of MAP kinase/ERK activity by maximally effective agonist concentrations in NG108-15 cells. In addition by using a continuous membrane potential assay, siRNA induced CRIP1a knockdown significantly increased anandamide-induced GIRK channel activation (p < 0.05) in AIT-20 cells. Conclusion: These studies provide evidence in vitro insight into the mechanisms involved in CRIP1a modulation of CB1 receptor signalling, a signalling system important for synaptic plasticity, neuroprotection, stress and anxiety and various other disease states.

POS-MON-050
BRAIN RESPONSES TO AIRWAYS IRRITATION IN INDIVIDUALS WITH COUGH HYPERSENSITIVITY

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Background: Cough hypersensitivity (CH) disorder is characterised by lowered cough reflex thresholds and persistent sensations of the urge-to-cough (UTC), suggesting dysfunction of components of the cough neural network. Aim: The purpose of this experiment was to investigate brain responses during inhalation of a tussive substance (capsaicin) in patients with CH and healthy controls to assess the contribution of changes in central processing to increased airways sensitivity. Methods: CH participants (n=16) and age and sex-matched controls (n=6) inhaled increasing doses of capsaicin until they coughed twice (C2 threshold) to determine their behavioural response. Blood oxygen level-dependent data was collected during capsaicin-evoked UTC challenges at C2 threshold in CH dose in CH participants. Control participants were scanned during inhalation of capsaicin dose equal to that given to their age and sex-matched CH participant to assess responses to comparable stimuli. Results: Behavioural data showed a significant decrease in the C2 threshold of CH participants compared to controls (t(18.25))=-2.32, p<0.05). Despite the disproportionate number of participants in groups, CH participants displayed increased activation bilaterally in insula and opercular cortices, thalamus and left orbitofrontal cortex during evoked UTC challenges at the same capsaicin intensity level as controls (p < 0.05). Conclusion: Cough hypersensitivity is associated with enhanced responses to capsaicin inhalation in brain regions that have previously been implicated in the representation of UTC. These data provide insights into dysfunction of the cough neural network in sensitised states and implications of this study for the management of CH will be dependent upon replication of these preliminary outcomes in a larger sample.

POS-MON-051
BINOCULAR RIVALRY AND SCHIZOTYPAL PERSONALITY TRAITS IN NON-CLINICAL INDIVIDUALS

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Binocular Rivalry occurs when different images are presented simultaneously to each eye. The perceptual competition of the two images results in the observer perceiving an alternation between the two distinct images. However, people also report periods of a mixed - with the images scrambled or fused - percept either for short or long period. Even if the mix percept is reported in studies of binocular rivalry, little is known about it. The mix percept appears to be longer and more frequent in early childhood and its existence proves that binocular rivalry is more than mere eye competitions. Clinical conditions show a atypical rate of perceptual alternation: bipolar disorder has a slower rate and anxious personality a faster one. Purpose: To show that mix percept might be correlated to clinical or subclinical conditions in particular with positive symptoms of psychosis characterized by hallucinations and perceptual aberration. Methods: Participants (n=70) performed a perceptual rivalry visual task viewing simultaneously and in each eye respectively a vertical gratings and a horizontal one. Participants reported which of the two images were dominant or if they perceive a mix percept.To measure several dimensions of schizotypy the Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE) was administered. Results: A Spearman’s rho correlation coefficient was computed to assess the relationship between the mix percept during binocular rivalry and the score obtained on the Unusual Experiences O-LIFE subscale. There was a positive significant correlation between the two variables (r(78)= .23, p = .045. Conclusion: The result of our research suggests that subjects with longer mix percept during binocular rivalry might be more at risk to develop psychological disorders. This could be useful in the clinical field to help early diagnosis of psychopathologies.

POS-MON-052
CORTICOCORTICAL CONNECTIONS OF AREA PGM IN THE MACAQUE MONKEY

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Purpose: The medial part of the macaque posterior parietal cortex (BA 7m, or PGM) has remained a relatively unexplored region, largely because of its inaccessible location. Recent imaging studies have ascribed a multitude of visuospatial and cognitive functions to the region, and this field is suggesting possible anatomical heterogeneity. The aim of this study was to examine variations of cortical projections to different sectors of PGM. Methods: 5 retrograde fluorescent tracers were placed in PGM in two macaque monkeys anesthetized with alfaxon (10 mg/kg). The distribution of labeled cells was visualized with fluorescence microscopy, and presented in computer-assisted reconstructions of the cortical surface. Results: The principal sensory input to PGM arrived from the high-order medial extrastriate cortices (V6A, and V2, and to a yet to be characterized area located adjacent to peripheral V2). Projections also originated in the oculomotor-related areas of the inferior parietal lobule (PG, Opt), in the lateral intraparietal area, and in the medial superior temporal visual area. Strong projections also stemmed from caudal cingulate area 23. Frontal projections arose mainly from the dorsal premotor cortex (F7), and from prefrontal areas 8 and 46. Rostral PGM received additional somatomotor input, from superior parietal areas PECi and V6Ad, whereas caudal PGM received stronger visual inputs. Detailed histological analysis confirmed the existence of two cytoarchitectonic variations within area PGM. Conclusions: The present results indicate that PGM receives consistent input from higher-order sensory, motor, and limbic cortices, in line with the proposed associative role of the area. At the same time, regional variations in the architectural structure and connectivity patterns likely reflect functional specializations within PGM.
POS-MON-053

LIVE IMAGING OF PEPTIDE-CELL INTERACTIONS IN TRIGEMINAL GANGLION THAT MAY CONTRIBUTE TO HYPERTENSION-ASSOCIATED HYPOALGESIA


Purpose: The association between high blood pressure and high pain tolerance is widely documented but poorly understood. Surprising impacts of this robust correlation include decreased risk of developing non-migrainous headache in populations with chronic hypertension. Mechanisms contributing to hypertension-associated hypalgesia may include interactions between peptides involved in the regulation of blood pressure (e.g. angiotensin II) and peptides involved in pain signal transmission (e.g. substance P). These pilot studies were undertaken to determine if high-sensitivity confocal microscopy techniques might enable direct imaging of these peptides binding to live cells within the trigeminal ganglia, which contain cell bodies of sensory neurons supplying the face and most of the cerebral circulation. Methods: We used high sensitivity confocal microscopy (Leica SP5 with avalanche photodiode detectors) to image angiotensin-II linked to Alexa 647 (AngII-A647, 100nM and 10nM) and substance P linked to Alexa 488 (SP-A488, 100nM and 10nM), in near real time, binding to live native neurons and blood vessels within acutely isolated trigeminal ganglia of mice (C57Bl/6, n = 10) and guinea pigs (n = 30). Results: AngII-A647 and SP-A488 were observed binding to membranes of neurons, Schwann cells and small blood vessels within trigeminal ganglia of both species. In many instances, binding of both peptide-fluorophore complexes to the same neuron or microvessel was observed. Conclusion: High sensitivity confocal microscopy enables direct visualization of fluorophore-labelled angiotensin II and substance P binding to live cells within trigeminal ganglion tissue from mice and guinea pigs. Further studies, using high speed resonant scanning, appear warranted to quantify peptide-cell binding and to determine if one peptide can influence the binding behaviour of the other.

POS-MON-054

EVIDENCE FOR MITOCHONDRIAL DNA DELETIONS IN VESTIBULAR HAIR CELLS OF THE AGED RAT

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Purpose: Falls by the elderly are surprisingly common with up to 75% of hospitalisations of elderly being due to falls. Impaired peripheral vestibular function is thought to contribute to these falls. Inner ear vestibular hair cells detect head motion and these cells are energetically demanding, in part due to the necessity to continuously remove calcium from the cell. Mitochondrial activity is critical for normal hair cell and vestibular system function. Mitochondrial DNA (mtDNA) encodes essential components of the mitochondrial respiratory chain. Ageing has been found to be associated with increased deletion mutation abundance in mtDNA and this has been shown to compromise respiratory chain activity. However, whether ageing causes an increase in mtDNA deletions in vestibular hair cells, potentially underlying age-related balance loss, is currently unknown. The aim of the present study was to characterise the effect of ageing on mtDNA deletion mutations in inner ear vestibular hair cells in the rat brain. Methods: Approximately 300 hair cells were laser microdissected from each of 14 young adult (4-6 months) and 7 old (23-26 months) male Fisher 344 rats. DNA was extracted and qPCR analyses carried out to compare the relative abundance of 4 mtDNA genes and mtDNA copy number between the two age groups. Results: There was a significantly higher age-related difference in abundance of CyB relative to ND1, ND4 and 12S mtDNA genes (p<0.001). There was no age effect on mtDNA copy number (p=0.44). Conclusions: These data indicate mtDNA deletions may contribute to age-related changes in vestibular system function.

POS-MON-055

RETINAL NOS IMMUNOREACTIVITY IS REDUCED IN FORM DEPRIVATION MYOPIA

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Purpose: Under abberant visual conditions, the eye grows too rapidly so that images are focused in front of the retina, causing myopia. Myopia can be induced in primates, mammals and birds when the eye is deprived of form vision (form deprivation myopia: FDM) or exposed to hyperopic defocus (lens induced myopia: LIM). Recent studies have described down regulation of nitric oxide synthase (NOS) in retinas of myopic eyes through LIM. Evidence supports some fundamental differences in mechanisms underlying myopia between FDM and LIM. Using a graded form defuser design, we investigated NOS immunoreactivity in form deprived eyes. Methods: Guinea pigs (n=31) wore filters on one eye from P6-P13. The filters were: a standard defuser (FFD: optical density=0.6); or one of three Bangerter foils (BF:0.8,0.6 or 0.4) that differed in cut-off of form vision (form deprivation myopia: FDM) or exposed to hyperopic defocus (BF0.8, BF0.6 and BF0.4). Results: AngII-A647 and SP-A488 were observed binding to membranes of neurons, Schwann cells and small blood vessels within trigeminal ganglia of both species. In many instances, binding of both peptide-fluorophore complexes to the same neuron or microvessel was observed. Conclusion: High sensitivity confocal microscopy enables direct visualization of fluorophore-labelled angiotensin II and substance P binding to live cells within trigeminal ganglion tissue from mice and guinea pigs. Further studies, using high speed resonant scanning, appear warranted to quantify peptide-cell binding and to determine if one peptide can influence the binding behaviour of the other.

POS-MON-056

HYDROGEN PEROXIDE ACTIVATES HIGH THRESHOLD INTRAMURAL VASCULAR MECHANORECEPTORS VIA TRPA1 BUT NOT TRPV1 CHANNELS IN THE GUINEA PIG BLADDER

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Purpose: It is known that reactive oxygen species can evoke bladder overactivity acting on capsaicin-sensitive afferent pathways in the bladder. However, it is still unclear which particular class of sensory neurons are activated and via which particular TRP channels. We determined the effect of hydrogen peroxide on the major classes of bladder afferents and the effects of TRPA1 and TRPV1 channel antagonists. Methods: Single unit extracellular recordings were made from pelvic nerves in flat sheet preparations of the guinea pig bladder in vitro in the presence of nicardipine (4μM). Results: Local or bath application of H2O2 (300-500μM) was found to activate the majority (70%) of capsaicin-sensitive mucosal and high threshold vascular afferents but not low threshold muscular or muscular-mucosal afferents. Bath application of H2O2 in mucosa-free preparations dose-dependently (30-1000μM) activated high threshold vascular mechanoreceptors (0.78±0.2 Hz at 300μM, n=10). TRPA1 antagonist, HC-030031 (10μM for 30min) inhibited H2O2-induced activation of vascular afferents by 64±9% (n=10). TRPV1 antagonist, capsazepine (10μM for 30min) did not affect action of H2O2 on vascular afferents (1±15%, n=10). Capsazepine (0.5μM for 30s) evoked activation of vascular afferents with mean firing of 3.6±0.7 Hz (n=12). The activation of vascular afferents after second application of capsaicin (after 30min of washing) was slightly reduced (by 16±5%, P<0.05, n=5). Capsazepine (10μM) abolished the effect of second application of capsaicin (by 99.6±1% (n=7). Conclusion: H2O2 dose-dependently activates majority of capsaicin-sensitive mucosal and vascular afferents but not low threshold stretch-sensitive mechanoreceptors in the guinea pig bladder. Activation of high threshold intramural vascular afferents by H2O2 is mediated via TRPA1 but not TRPV1 channels.
POSTERS

Monday

POS-MON-057
GTF2IRD1, A GENE IMPLICATED IN THE AUDITORY PATHOLOGY OF WILLIAMS-BEUREN SYNDROME

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Williams-Beuren Syndrome (WBS) is a genetic condition caused by a hemizygous deletion involving 28 genes (7q11.23). WBS children are typically reported as having hyperacusis (highened sensitivity to sound) or auditory allodynia (aversion to certain sounds). However, high-frequency sensorineural hearing loss (SNHL) is common in WBS. GTF2IRD1 is a transcription factor encoded by a gene located within the deleted region and a prime candidate for the cause of several neurocognitive features of WBS. Purpose: To investigate the role of GTF2IRD1 in WBS hearing loss and to identify the cellular and molecular cause. Methods: Using a LacZ knock-in reporter, we analysed the expression of Gtf2ird1 in the inner ear. Then, using Gtf2ird1 null mice, we examined hearing capacity by auditory brainstem response (ABR), which assesses sound-evoked auditory neurotransmission from the cochlear nerve to the auditory midbrain, and by distortion product otoacoustic emissions (DPOAE) to analyse the amplifying function of the outer hair cells. Results: Gtf2ird1 expression was detected in the cochlear sensory hair cells, spiral ganglion neurons, and in the vestibular system. Gtf2ird1 knockout mice showed higher auditory thresholds in both ABR and DPOAE hearing assessments (4-32 kHz; n=15; P<0.05). No anatomical abnormalities in neural fibers connecting hair cells to the spiral ganglion neurons were observed by neurofilament-200 staining. Conclusion: Gtf2ird1 is expressed in the sensory neural tissue of the organ of Corti and absence of Gtf2ird1 led to reduced hearing function. Our data supports Gtf2ird1 contribute to sound transduction and auditory neurotransmission and thus GTF2IRD1 deletion in WBS likely leads to the manifested hearing phenotype.

POST-MON-058
SUBMODALITY CONVERGENCE IN PRIMARY AND SECONDARY SOMATOSENSORY AREAS OF THE CAT

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Purpose: The functionally distinct populations of mechanoreceptive afferents remain largely segregated along the somatosensory pathway. Recent studies have indicated that this segregation may not remain as strict in neurons of the primary (S1) and secondary (S2) somatosensory regions of the cortex. We investigated the extent to which there is submodality convergence of functionally distinct afferent populations in S1 and S2 cortical regions in the cat. Methods: Sinusoidal vibratotile stimuli of 20Hz (or 23Hz), 200Hz, or combined 20/200Hz (or 23/230Hz), at various amplitude combinations, superimposed on a step indentation, were presented to the glabrous skin of the forepaw of anesthetized adult cats (n=9). Multi-unit spike activity was recorded using 100-channel Utah and 64-channel NeuroNexus arrays inserted into contralateral S1 and SII, in the region receiving input from the glabrous skin of the forepaw. All procedures were approved by the Ethics Committee of UNSW. Results: Multi-unit activity showed facilitating responses whereby the activity under the combined stimulus was greater than the sum of the activity produced by both the low and the high frequencies alone. The proportion of the facilitatory responses was greater in S2 (11%) than in S1 (2%). Conclusion: Our results provide further evidence that neurons as early as primary and secondary somatosensory regions of the cortex show submodality convergence and are not responsive to a single modality as originally proposed.

POS-MON-059
AGEING EFFECTS ON AUDIOVISUAL SYNCHRONY: JUDGEMENTS AT SCALABLE DECTIBILITY

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Purpose: A previous study suggests that the time window during which auditory and visual stimuli appear synchronous is wider in older adults (Hay McCutcheon et al., 2009). Our study aimed to determine whether the audio-visual synchrony window differs between age groups: 1) once stimuli are individually scaled according to detection thresholds, 2) once criterion effects are minimised by using a two-interval-forced-choice (2IFC) paradigm, and 3) for both low (500 Hz) and higher sound frequencies (4000 Hz– where prestecysis results in elevated audiotric thresholds). METHODS Audiovisual asynchronization judgments were measured on ten older (64–72 years old) and twelve younger (21–32 years old) adults for visual (Gabor, 10ms, 3c/deg, centrally-fixated) and auditory (pure-tone pip, 10ms, binaural, via headphones, presented on a 75dB pure-tone mask) stimuli, using a 2IFC method. A 3-down-1-up staircase measured sound-lead and sound-lag asynchrony thresholds at approximately 75% correct responses. Individual psychometric functions (fitted with cumulative Gaussians) for contrast and pip detection were first obtained. To match stimulus detectability between age groups, auditory and visual stimuli were presented at threshold+10 sigma (sigma–standard deviation of the psychometric function fit). RESULTS Both age groups had similar sound-lag asynchrony threshold but the older adults had a significantly earlier sound-lead threshold than the younger adults (RM-ANOVA; p=0.02), which was not dependent on sound frequency (p=0.7). There was a trend for wider windows in the older adults but did not reach statistical significance (500 Hz/4 kHz – older: 338±23/325±18ms (mean±95% CI), younger: 392±72/370±78ms; p=0.14) CONCLUSION Older people show differences in audiovisual synchrony perception. When auditory stimuli are presented before visual stimuli, older adults are more likely to judge the stimuli as synchronous, even at scaled detectability. This phenomenon is not specific to higher-frequency stimuli where older people have reduced sound detection capacity.

POS-MON-060
SCALING UP THE PRIMATE CEREBRAL CORTEX: PATTERNS AND KEY AREAS OF EXPANSION ACROSS SPECIES

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Purpose: The cerebral cortex of different primate species share a common architecture. However, there are vast differences in brain size across species and it is clear that larger brains aren’t simply linearly scaled up versions of smaller brains. Here we use computational methods to quantify the regions of high expansion and compare the topography of cortical subdivisions. METHODS: Surface based atlas models of the marmoset (n=3) and macaque (n=3) monkey cortex were registered using the software package CARET and the spherical landmark vector difference algorithm. The registration was constrained by the location of homologous cortical subdivisions. Expansion was quantified as the ratio of the area of the corresponding polygons on the macaque and marmoset model meshes. Results: We found a high degree of expansion in the lateral prefrontal cortex, the temporal parietal junction and intraparietal sulcus. The expansion maps are well correlated with previously published macaque to human registrations, suggesting there is a general pattern of primate cortical scaling. The correspondence of cortical subdivisions was reasonably good for putative homologues, except near areas of high expansion, where some subdivisions had been displaced. Conclusion: We found evidence that key areas of the primate cortex have expanded disproportionately with increases in brain size, and that the pattern of expansion is similar across marmosets, macaques and humans. In future studies, these coregistered models may be useful for mapping connectivity and functional data from one species to another for further interspecies comparisons.
POS-MON-061

CONTEXTUAL EFFECTS IN SPEED TUNING OF NEURONES IN THE MIDDLE TEMPORAL AREA (MT)

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Purpose: An important issue in understanding visual motion processing is whether neurons can signal the speed of a moving object accurately, irrespective of the spatial frequency content of the image. Studies in which the spatial and temporal frequencies of drifting gratings were manipulated independently have concluded that only a minority of neurons are truly ‘speed-tuned’. However, in most natural situations objects move as wholes, occupying successive locations across the retina, rather than occupying a constant location, like drifting gratings. Here, we investigated whether the degree of speed-tuning of cells in area MT varies according to the mode of stimulus presentation.

Methods: Marmosets were anaesthetised with sufentanil (8 μg/kg/hr) and N2O (70% in oxygen), and the eyes were focused on a computer screen where visual stimuli (Gabor patches of variable spatial frequency) were projected. Recordings from 121 cells compared the responses to two types of visual stimulus: a ‘moving Gabor’, versus the traditionally used ‘stationary Gabor’ (in which only the grating elements drift, over a constant region of space).

Results: Across the same population of cells, a significantly higher percentage of speed-tuned neurons was found when the ‘moving Gabor’ stimulus was used, versus the ‘drifting Gabor’ (p<0.001). The preferences for spatial frequency and speed changed, with the use of ‘moving Gabor’ generally resulting in selectivity for lower spatial frequencies (p<0.001), and higher speeds (p<0.05), in comparison with ‘drifting Gabor’.

Conclusion: By adopting a more naturalistic mode of stimulus presentation, we detected a higher proportion of speed-tuned cells in MT. These findings suggest that future studies trying to determine speed-related properties should consider adopting stimuli that more closely resemble natural motion.

POS-MON-063

EFFECTS OF AGE ON THE PRESERVATION OF RESIDUAL HEARING WITH COCHLEAR IMPLANTS

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Background: Cochlear implant (CI) recipients with residual hearing in their implanted ear receive both electric and acoustic stimulation. As a result, optimal hearing preservation is a major factor in achieving the best possible auditory experience for these patients. However, a small but significant number of CI patients lose their residual hearing following implantation, although the mechanisms of this loss are not clear. Methods: To determine the effects of age on the preservation of residual hearing, this study evaluated hearing preservation, using auditory brainstem response recordings, in both neonatal (n=8) and adult onset (n=3) partially deaf cats. All animals received approximately 6 months intra-cochlear electrical stimulation from a clinical CI and speech processor, after stabilisation of their systemic aminoglycoside induced partial hearing loss. Results: All animals had measurable residual hearing in the low- to mid-frequency range (below 8 kHz, apical to the intra-cochlear electrode array) throughout the experiment. There was no significant loss of hearing in the neonatally deafened animals during the chronic stimulation period (paired T-test, p = 0.13). In contrast, the adult onset group exhibited a significant hearing loss of approximately 8 dB (paired T-test, p = 0.03). Conclusions: These results indicate that CI use per se, does not result in a significant loss of residual hearing (i.e. no change in threshold for the neonatally deafened group). However, age may be a factor in the preservation of residual hearing with CI use, as a small loss was observed in the adult onset group. These results need to be confirmed with a larger sample size, and confounding factors (such as the total duration of deafness) also need to be further examined.

POS-MON-064

INFLUENCE OF IMAGE TEXTURE ORIENTATION RELATIVE TO MOTION DIRECTION ON RESPONSE OF PRIMATE AREA MT NEURONS AND HUMAN PERCEPTION

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Purpose: We investigate how responses of neurons in marmoset MT and human perception depend on the orientation (and bandwidth) of image textures relative to their direction of motion. Methods: Extracellular single-unit recordings were made from area MT of anaesthetised (sufentanyl-forte, 9 μg/kg/hr) marmosets (n = 2). The stimulus was a texture with varying orientation bandwidth. Textures moved orthogonal, parallel or 45 degrees relative to the dominant orientation. Neurons were classified as ‘pattern cells’ or ‘component cells’ using standard techniques. Human observers (n = 7) reported the perceived motion direction of the same stimuli. Results: Human participants accurately reported the correct motion direction when textures moved orthogonal or parallel to their dominant orientation. For oblique motion, participants reported directions intermediate to the true direction and that orthogonal to the dominant orientation. Neuronal responses depended on cell class. In 5 pattern cells direction-tuning curves were unimodal for all textures. Preferred direction was the same for parallel and orthogonal motion direction, but for oblique was intermediate to the true motion direction and that orthogonal to the dominant orientation. In 8 component cells, responses were strong and unimodal for orthogonal motion, but weak and bimodal for parallel motion. For oblique motion, direction tuning curves were aligned to the dominant orientation of the textures. Conclusion: Response of pattern and component cells was similar for oblique and orthogonal motion but different for parallel motion. In human observers, oblique motion leads to characteristic inaccuracies in motion computations. Response of pattern cells to parallel motion was consistent with the reports of human observers.
POSTERS Monday

POSTER - MON-065

DISTRIBUTION OF SENSORY FIBRES CONTAINING CGRP BUT NOT SUBSTANCE P IN SKELETAL MUSCLES OF MICE

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Purpose: Calcitonin gene-related peptide (CGRP) and substance P (SP) are the two peptides principally involved in primary nociceptor transmission. CGRP generally co-exists with SP in unmyelinated primary nociceptors, where it serves to facilitate the release and inhibit the degradation of SP. However, previous studies have observed a population of medium sized primary afferents that express CGRP but no other neuropeptides. Therefore, it is possible that CGRP has functions beyond modulating the effects of SP, playing a role in sensory processing that is yet to be discovered. This project aims to observe patterns in the expression of CGRP and SP in sensory nerve fibres in murine skeletal muscle. Methods: Gastrocnemius and erector spinae muscle from C57/B16 mice (n = 7) was fixed in Zambon’s solution, sectioned at 12 µm, and processed for multiple labeling immunohistochemistry. Tissue was labeled with antibodies against neuron specific enolase (NSE), a pan-neuronal marker, CGRP and SP. The distribution of CGRP and SP immunoreactive fibres was quantified using 3 categories: fibres in connective tissue, fibres travelling alongside blood vessels, and fibres extending through muscle tissue with no obvious association with other structures (termed “free fibres”). Results: The proportion of fibres containing CGRP alone and CGRP with SP does not vary significantly between gastrocnemius and erector spinae muscles (p = 0.485). Of free fibres containing CGRP, 64% did not express SP. 52% and 36% of CGRP positive fibres surrounding blood vessels and in connective tissue respectively contained CGRP without SP (X² = 14.5, p = 0.001). Conclusion: In murine muscle, nerve fibres immunoreactive for CGRP but not SP show strong preferential distribution to free fibres, and occur less frequently in fibres surrounding blood vessels. This distribution is consistent with these fibres being high threshold mechanoreceptors.

POSTER - MON-066

REMODELLING IN ADVANCED RETINAL DEGENERATION IN RD1 MICE

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Grefeath U1, O’Brien EE1, 2 and Fletcher EL 1Department of Anatomy and Cell Biology, 2Department of Electrical and Electronic Engineering, University of Melbourne. Purpose: Retinitis pigmentosa (RP) refers to a family of inherited diseases that lead to blindness. In mid to late stages of the disease when most photoreceptors have been lost, significant remodelling of inner retinal neurons occurs. Since most patients are targeted for restorative treatments in these advanced stages of disease we aimed to characterise the cause and progression of events during late phase degeneration in a mouse model of RP, the rd1 mouse, which carries one of the human mutations. Methods: We used two double mutant transgenic mice, 1. rd1-FTL mouse, containing the rd1 mutation and an axon-targeted β-galactosidase (β-gal) reporter system under the regulation of the c-fos gene. In these mice neurons that express c-fos also express β-gal in the entire cell. 2. rd1-Thy1-YFP mice which in addition to rd1 express YFP under the control of Thy1 promoter in a subpopulation of ganglion cells. Retinal tissue from mice between postnatal days P30 to P365 (5 per timepoint) were immunocytochemically analysed as wholemounts or cryostat sections. Results: From P120 a strong upregulation of β-gal in the central retina was observed, which was independent of light, that spread to cover the entire central retina (X² = 14.5, p = 0.001). Staining was found in amacrine, ganglion, and Müller cells. Notably, Müller cells showed a loss of glutamine synthetase and Kir 4.1 immunoreactivity and an upregulation of cyclin D1 in those regions of high β-gal labelling and ganglion cells show altered morphology and stratification of their dendrites. Conclusion: We propose this significant upregulation of β-gal activity may reflect active cellular signalling that indicates inner retinal remodelling. The progression of remodelling events could be linked to the pattern of glial cell dysfunction.

POSTER - MON-067

OPTIMAL WAVEFORM PARAMETERS FOR EXTRACELLULAR ACTIVATION OF RGCS

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Purpose: Retinal prostheses seek to restore visual perception in the blind through electrical stimulation of surviving retinal ganglion cells (RGCs). While increased stimulus amplitude often results in greater neural activity, the influence of other waveform parameters such as phase duration on efficacy is unclear. We wished to determine the waveform parameters most effective in activating RGCs using planar electrodes fabricated from nitrogen-doped diamond. Methods: We made whole-cell patch-clamp recordings from 41 A2-type RGCS in retinal whole mount preparations from 26 Sprague-Dawley rats. Membrane potential recordings were amplified (NPI, BA-1S), sampled at 50kHz and stored in digital form. Cells were stimulated using a diamond electrode (200x200um) positioned over the soma and driven by current injection (Multichannel Systems, MCS-4004). Stimuli were delivered against a distant monopolar return electrode. Results: Phase duration and polarity had the largest effect on activation thresholds - cells were most responsive to cathodic electrode. Confirming earlier results using single electrodes, we found that receptive fields in DM were larger than those in V2, and that DM comprises a representation of the upper visual field that is adjacent to V2. The response onset latencies measured at center of the receptive fields were 48±19 ms in V2, compared to 64±15 ms in DM. Orientation tuning bandwidths, measured with gratings, were similar in DM and V2 (mean, 68.5±35 degrees). Conclusion: Current technologies already allow high-throughput characterization of neuronal response properties across many sites, including multiple visual areas. When combined with algorithms currently under development for online unit sorting, these automated routines will allow an unprecedented level of insight on sensory processing by neuronal populations.

POSTER - MON-068

SIMULTANEOUS MAPPING OF RECEPTIVE FIELD PROPERTIES OF LARGE NEURONAL POPULATIONS IN EXTRASTRIATE CORTEX

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Purpose: Traditional approaches to characterization of responses in visual cortex have relied on sequential sampling of single cells, or small populations. However, the usefulness of brain-machine interfaces is likely to depend on more sophisticated approaches, including simultaneous monitoring of activity of large populations of neurons. Here we demonstrate the feasibility of stable recordings from up to 96 multunitis across the second [V2] and dorsomedial [DM] visual areas. Methods: Marmosets were ariaeathetized with sufentanil (8 µg/kg) and N2O (70% in oxygen), and the eyes were focused on a computer screen where visual stimuli were projected, over an area encompassing more than 40° of the visual field. Recordings were obtained through 96-channel “Utah” arrays, inserted in dorsal extrastriate cortex, including parts of areas V2 and DM. Results: Following a period of stabilization of several hours, high signal-to-noise ratio responses could be obtained from all recording channels. Confirming earlier results using single electrodes, we found that receptive fields in DM were larger than those in V2, and that DM comprises a representation of the upper visual field that is adjacent to V2. The response onset latencies measured at center of the receptive fields were 48±19 ms in V2, compared to 64±15 ms in DM. Orientation tuning bandwidths, measured with gratings, were similar in DM and V2 (mean, 68.5±35 degrees). Conclusion: Current technologies already allow high-throughput characterization of neuronal response properties across many sites, including multiple visual areas. When combined with algorithms currently under development for online unit sorting, these automated routines will allow an unprecedented level of insight on sensory processing by neuronal populations.
PHASE SENSITIVITY OF COMPLEX CELLS INCREASES AT LOW CONTRASTS

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Purpose: Neurons in primary visual cortex are classified as either simple or complex. The hierarchical model proposed that the luminance phase-insensitive receptive fields (RFs) of complex cells could be formed by combining input from multiple simple cells with similarly oriented but spatially offset receptive fields (Hubel and Wiesel, 1962). Complex cells can become phase-sensitive following adaptation or as contrast is reduced (Crowder et al. 2008). Modulation of complex cell responses at low contrasts is consistent with the hierarchical model (Van Kleef et al. 2010). It is possible that phase-sensitive responses of some complex cells at low contrasts could be due to differences in the contrast response threshold of afferent simple cells: at low contrast, complex cell responses could be due to the phase-sensitivity of the complex cell due to the phase-sensitivity of the remaining super-threshold inputs.

Methods: We examined this hypothesis by quantifying the phase-sensitivity of complex cells as a function of contrast. We recorded extracellular spiking responses from 62 neurons in cat visual cortex (area 17 and 18) while presenting stationary contrast reversing sine-wave gratings at multiple spatial phases and contrasts. Results: We show that a majority of complex cells (79%; p<0.0001) show a significant increase in their phase-sensitivity at low contrast relative to high contrast. Conclusion: Reducing stimulus contrast below the threshold of a subset of complex cell inputs increases the phase-sensitivity of the complex cell due to the phase-sensitivity of the remaining super-threshold inputs.

DIRECT INHIBITION MODIFIES THE SHARPNESS OF SPATIAL-TUNING CURVES AND RESPONSE OUTPUT OF GANGLION CELLS

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In the central nervous system, neuronal responses depend on the balance between excitatory and inhibitory synaptic inputs. Purpose: Our aim is to understand how this balance determines retinal ganglion cell output. For this, we first examined the impact of changes in the ratio of excitation and inhibition of spatially tuned synaptic conductances on neuronal responses. Secondly, we tested the effects of various levels of inhibition on neuronal output while keeping a constant level of excitation. Thirdly, we studied the effects of the relative timing of inhibition with respect to excitation on spike responses. Methods: We carried out dynamic clamp recordings from ganglion cells in whole-mount retinae of the mouse using three sets of synaptic conductances: 1) Light-evoked excitatory and inhibitory conductances recorded in response to different stimulus sizes were injected and their ratio was varied. 2) The magnitude of inhibition was changed while keeping the magnitude of excitation constant. 3) While keeping the amplitude of excitation and inhibition constant, the time difference between the onset of excitation and inhibition was varied. Results: Injection of balanced excitation and inhibition (ratio 1:1) generated area response functions typical of physiological responses displaying surround inhibition. Increases in the level of inhibition resulted in more prominent surround inhibition (n=22). When excitation and inhibition were coincident, neuronal responses decreased as the level of inhibition increased (n=10). Inhibition had the strongest impact when it preceded excitation and the longer it lagged excitation, its effect became less pronounced (n=12). Conclusions: Our results suggest that the balance of excitation and inhibition plays an important role in sharpening the spatial-tuning of ganglion cells. In addition, the relative timing and amplitude of excitation and inhibition are critical for determining the strength of ganglion cell responses.

THE P2X4 RECEPTOR AND NTPDASE1/CD39 ENZYME ARE EXPRESSED IN THE MOUSE RETINA

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Purpose: ATP and other related purines have been found to act as transmitters at a wide range of purine receptors. Activation of purine receptors has been shown to be important for signaling between neurons and glial cells in the retina. However, the exact cellular expression of some purine receptor subtypes in the retina remains to be elucidated. The aim of this study was to characterise the expression of purinergic system components, the P2X4-receptor and NTPDase1, in the wild-type (WT) mouse retina. Methods: Immunohistochemistry was used to assess the cellular localisation of the ATP receptor, P2X4, and the enzyme responsible for ATP hydrolysis, NTPDase1, in retinae of adult WT (C57Bl6/J) mice (n=8). Results: P2X4-R immunoreactivity (IR) was present as discrete puncta within the retinal synaptic layers and colocalised with neurons important for lateral inhibition, the horizontal and amacrine cells. Additionally, P2X4-R-IR was closely associated with bipolar and ganglion cells and Müller glial cells. Both P2X4-R-IR and NTPDase1-IR was apparent on microglia in the WT retina. Conclusion: The P2X4-R may be involved in modulating synaptic function and also modulating microglial immuno-surveillance in the healthy retina. By controlling extracellular levels of ATP, NTPDase1 expressed on microglia may modulate the cellular immune response as well as contribute to neuronal and glial signalling in the mouse retina.

FUNCTIONAL DIFFERENCES BETWEEN TWO CLASSES OF LAYER 2 PRINCIPAL NEURONS IN THE PIRIFORM CORTEX IN VIVO

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Semilunar (SL) and superficial pyramidal (SP) cells form the main input layer (layer 2) of the piriform (primary olfactory) cortex. Previous work has shown that SL and SP cells differ significantly in their morphology, somatic lamina position, connectivity and electrophysiological properties measured in vitro. Purpose: This study had two aims: i) to determine whether previously observed differences in SL/SP cell electrophysiology are maintained in vivo; ii) to investigate whether SL and SP cells respond differently to odours in vivo. We hypothesised that SP cells, with their more abundant intracortical connectivity, would respond more broadly to odors than SL cells. Methods: The anterior piriform cortex was surgically exposed and whole-cell recordings were made from layer 2 principal neurons. A palette of up to 15 monomolecular odorants was applied using a computer-controlled olfactometer. Results: Of several electrophysiological parameters, only the amplitude of the spike afterhyperpolarisation showed the expected correlation with the somatic lamina depth of the neuron (r²=0.13, p<0.05, Pearson analysis). It is likely that many electrophysiological signatures of SL and SP cells are obscured in vivo by abundant synaptic activity. Nevertheless, our data indicate that somatic lamina depth is a proxy for cell identity. Our preliminary data also support a difference in the olfactory tuning between these two types of neurons. Putative SL cells responded to fewer than 3 of up to 13 odorants (n=7), whereas putative SP cells responded to more than 3 of up to 7 odorants (n=4). Conclusion: Our data suggest that SL and SP cells may perform distinct roles in the processing of olfactory information.
POS-MON-073
THE ANATOMY OF HONEYBEE OCELLI
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Purpose: Honeybees, like most flying insects, have a visual system composed of three ocelli (simple eyes) located on the top of the head, in addition to two large compound eyes. Although numerous experiments have been conducted to investigate the role of the ocelli within the visual system, their exact function remains controversial. Methods: In this study, the three-dimensional structures of the honeybee ocelli are mapped with the aim of assisting in determining the role of the ocelli in that species. I investigate the morphological and optical characteristics of honeybee ocelli using scanning electron microscopy, semi-thin sections and focal length measurements of both the median and lateral ocelli. Three-dimensional reconstructions of honeybee ocellar lenses and retinas were also generated from serial sections of the ocelli. Each type of ocellus has two retinas, the dorsal and ventral. Results: The study assesses for the first time the spatial resolution of both retinas using the hanging drop technique. By using the 3-D model that is presented, it was possible to determine the visual fields of the various retinas. The dorsal retina views the horizon while the ventral retina views the sky, suggesting quite different roles in attitude control. It was found that the dorsal retina has a higher spatial resolution than the ventral retina in both ocellar types, consistent with the need to assess spatial detail on the horizon. The lateral ocellus was also found to have a higher resolution than the median ocellus.

POS-MON-075
COLD-SENSING NERVE TERMINALS IN THE CORNEAL EPITHELIUM ARE TRPM8-IMMUNOREACTIVE AND HAVE A COMPLEX MORPHOLOGY
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The aim of this study was to determine nerve terminal morphology of functionally defined cold-sensing neurons. We used small-diameter suction electrodes (≈50µm) applied to the surface of the guinea pig cornea to record nerve impulses originating in single sensory nerve terminals. Cold-sensing nerve terminals were identified by the presence of ongoing activity (2–15 Hz) that was increased by cooling (to 28 °C) and decreased by warming (to 37 °C). We marked the recording site by application of Fast Blue through the suction electrode at the end of our recordings, and then used whole-mount immunohistochemistry to double label nerve terminals under the electrode with antibodies directed against β-Tubulin (pan neuronal marker) and TRPM8 (cold-sensing ion channel). Confocal microscopy was used to image nerve terminals through the entire thickness of the corneal epithelium and explore their morphology in 3D. We successfully identified a single TRPM8-immunoreactive nerve terminal complex under the electrode in all five of the recordings we have made to date. Axons forming these terminals arose from sub-basal nerves at the base of the epithelium and formed a cluster of highly branched fibres that terminated in both the wing and squamous cell layers. Each of the many branches in these complex terminal structures had multiple bulbous endings. All TRPM8-immunoreactive nerve terminals in the cornea had this same complex morphology. β-Tubulin-immunoreactivity revealed that many of the surrounding nerve terminals had different morphologies and lacked TRPM8-immunoreactivity. These findings demonstrate that cold-sensing nerve terminals in the corneal epithelium can be distinguished on the basis of their morphology and neurochemistry.

POS-MON-074
INERVATION OF PERIPHERAL AND CENTRAL AUDITORY TISSUES BY HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS IN VITRO
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Purpose: The loss of sensory hair cells and auditory neurons that occurs as a result of hearing loss is irreversible. Progressive degeneration of auditory neurons in severe-to-profound deafness is thought to perturb the effectiveness of cochlear implants. Stem cell therapy to regenerate auditory neurons thereby offers a potential to restore hearing in patients with severe deafness. We have previously shown that neurons derived from human embryonic stem cell (ESCs) by treatment with Noggin and Y27632 can innervate early postnatal rat cochlear explants. This project aims to investigate peripheral and central synapse formation between ESC-derived neurons and target tissues in vitro. Methods: Human ESC-derived neurons were co-cultured with their peripheral target tissue, sensory hair cells, from postnatal day 1-2 rats (n=60), or with their central target tissue, cochlear nucleus slices, from postnatal day 9-12 rats (n=8) in vitro for 2-3 weeks. Synapse formation was examined using immunofluorescence and confocal microscopy. Results: Human ESC-derived neurons innervated the rows of sensory hair cells in all co-cultures. Immunoreactivity to pre-synaptic markers synapsin 1 and vesicular glutamate transporter-1 was found near the site of innervation. Numerous synapsin 1-positive neurites of sensory neuron-like cells also innervated cochlear nucleus slices. Conclusion: Human ESC-derived neurons are capable of innervating both peripheral and central targets in vitro, and these newly formed synapses contain pre-synaptic markers, and the correct glutamatergic phenotype. This assay will be used to investigate the timing and characteristics of synaptogenesis between ESC-derived neurons with their peripheral and central targets in vitro, with a view to determining whether this can improve cochlear implant performance in future.

POS-MON-076
THE EFFECTS OF CATHODAL TRANSCRANIAL DIRECT CURRENT STIMULATION (c-tDCS) ON MODIFICATION OF SENSORY AND PAIN PROCESSING: A SYSTEMATIC REVIEW AND META-ANALYSIS
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Purpose: Cathodal transcranial direct current stimulation (c-tDCS) reduces pain by modifying neurons firing rates. The current study evaluates the effects of c-tDCS on sensory and pain threshold (STh and PTh) in healthy individuals, and pain level (PL) in patients with chronic pain. The secondary aim is to find c-tDCS optimal parameters for its maximal effects. Method: seven electronic databases were searched for the studies on the effects of c-tDCS compared to controls. Studies in which measured STh, PTh, and PL by numeric or visual analogue scale were included. Methodological quality of included studies was examined using PEDro and Downs and Black (D&B) assessment tools. Results: Data from 2 studies on chronic pain revealed significant decrease in PL following application of c-tDCS on cortex (P=0.03). Studies investigating STh and PTh in healthy individuals demonstrated significant increase in STh with both c-tDCS application over S1 (P=0.00001) and M1 (P=0.00001). C-tDCS was effective on PTh only when S1 was stimulated (P<0.00001). Identical c-tDCS parameters were used in all trials. Discussion: The result indicates that c-tDCS is a non-invasive neuromodulatory technique which could be used for treatment of chronic pain. The available evidence suggests that application of c-tDCS on S1 can increase both STh and PTh in healthy individuals, while its application on M1 can only increase STh. Due to small number of included studies and small sample size in these studies interpretation of the results should be considered cautiously.
**POSTERS-MON-077**

**IS THERE A RELATIONSHIP BETWEEN ORIENTATION BIAS OF THE GENICULATE AFFERENTS AND THE ORIENTATION SELECTIVITY OF THE CELLS THEY PROJECT TO IN THE CAT STRIATE CORTEX?**

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**Purpose:** To study whether orientation biases of single geniculate afferents can explain the orientation selectivity of the neurons in the striate cortical orientation domain that they terminate in. **Methods:** We used optical imaging of intrinsic signals to obtain a map of the orientation domains on the dorsal operculum of the primary visual cortex of the anaesthetised cat. We then applied 10mM Kainic acid solution for 12 hours (67 μl/hr) over the exposed visual cortex, which is known to suppress all cortical cell activity but leave the afferent fibres in the region still functional. Electrophysiological recordings with high impedance tungsten electrodes were then carried out to record action potentials from single geniculate afferents in one of the larger orientation domains. The recorded units were identified as geniculate fibres if they were driven by only one eye, had fast-rising spike and spontaneous activity higher than the average striate cell. **Results:** Response of each of the recorded fibre (n = 9) was only biased for stimulus orientation, with the preferred orientation index difference between optimum and orthogonal orientations/response in optimum) for these putative afferents being 0.39±0.06. However, the orientation preferences of 8 of the 9 units fell within 20° of the preference of the corresponding orientation domain. **Conclusions:** The strong relationship between the orientation preference of the geniculate fibres and the cells in the same part of the striate cortex suggests that the preferred orientation of an orientation domain can be determined by the orientation bias of the geniculate afferents that feed into that domain.

**POSTERS-MON-078**

**SEGMENTAL DEPENDENT DIFFERENCES IN NEUROCHEMICAL PROFILES OF CALCITONIN GENE-RELATED PEPTIDE CONTAINING SENSORY NEURONS**

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**Purpose:** Many small diameter nociceptive neurons in dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). In mouse C7 DRG, a population of yet uncharacterised mid-sized neurons express CGRP without SP. Using multiple labelling immunohistochemistry we further neurochemically characterised these neurons and compared their presence in DRG of representative spinal segments. **Methods and Results:** Cervical (C7), thoracic (T5), lumbar (L4) and sacral (S3) DRG were removed from C57/B16 mice. Free-floating sections were labeled for CGRP, SP, and NF200 or TRPV1. About 50% of CGRP neurons did not express SP (CGRP+SP-; n=6) and there was no significant difference in this proportion between DRG of different spinal segments (p=0.816). Significantly more CGRP neurons expressing SP (CGRP+SP+) also contained TRPV1 compared to CGRP+SP- neurons (95% and 24%; n=4; p<0.0001). The proportion of CGRP neurons that expressed TRPV1 was similar between DRG of different spinal segments (p=0.957). Preliminary results also show that more CGRP+SP- neurons also expressed NF200 the CGRP+SP+ neurons (84% and 19%; n=1). There was a significant difference between the average size of the CGRP+SP- neurons and CGR of different spinal segments. In C7 (n=4) and L4 (n=2) DRG CGRP+SP- neurons were on average larger (mean soma size 471.5±17.9µm2 and 432.1±45.7µm2) compared to T5 (n=2) and S3 (n=2; 319.5±8.4 µm2 and 313.5 µm2; p=0.0123) DRG. **Conclusions:** Neurons expressing CGRP without SP are a significant population present in DRGs from different spinal cord levels. The increase in average size of CGRP+SP- neurons of the cervical and lumbar segments may correspond to higher number of mechanoreceptors from these segments.

**POSTERS-MON-079**

**SPECTRAL SENSITIVITY AND OPSIN SEQUENCES IN A PARROT SPECIES SHOWING EXTREME INTRASPECIFIC PLUMAGE COLOUR VARIATION**

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**Purpose:** The production of the neural impulses that serve vision is dependent on the retina photoreceptor responses to different wavelengths of light. Specifically, changes in the amino acid sequence of opsins in the light-sensitive visual pigments can cause shifts in wavelength sensitivity. The 25 parrots use ultraviolet cues for some aspects of visual behaviour. The 25 amino acid extension in the rod opsin is, to our knowledge, unique in an opsin that is otherwise highly conserved among all vertebrates. The extension also makes the opsin sensitive to ultraviolet light, making P. elegans an ideal potential candidate for intraindividual variation in retinal physiology, hitherto unreported in birds. **Methods:** Using microspectrophotometry, we measured the responses of individual retinal photoreceptors to obtain spectral sensitivity of wild-caught individuals of the primary visual cortex (V1) in early life allows for better recovery than an identical injury sustained in adulthood. However, the cellular / aural anatomical changes in the extrastriate cortex and visual thalamus associated with this phenomenon have yet to be characterised. **Methods:** Using the complex visual system of the marmoset monkey (Callithrix jaccus) as a modality, we examined changes in neonates and adults following partial unilateral ablation of V1. Ablations were performed in area V1 in the neonatal postnatal day (PD) 14 (n=2), and adult (>2 years, n=2) marmosets. Animals were allowed long-term recovery before undergoing transcendal perfusion for immunohistochemical analysis. Serial coronal 40µm sections were cresyl violet and myelin silver stained or immunolabelled with nonphosphorylated neurofilament (NF), calsykalin (Cytb) and parvabumin (PV) to identify neuronal populations in areas V1, V2, V3, the middle temporal area (MT), the dorsal medial area (DM), and the dorsal lateral geniculate nucleus (dLGN). Volumes of areas were then quantified using Cavalieri’s method on Fiji. **Results:** Furthermore, the present study suggests a reduction in NFN average throughout all laminae in area MT in the lesioned hemisphere in adult lesioned animals yet MT in neonatal lesioned remains comparable to that of the control. An increase in density in Cb+ cells in layer 2 of the residual V1 compared to contralateral V1 can be observed in both cohorts as well as a volumetric decreases in the contralateral dLGN. **Conclusion:** Unilateral lesion to V1 has a significant affect on the thalamus and extrastriate areas of the visual cortex, in both hemispheres for both neonates and adult animals. Understanding the specific anatomical changes following a lesion of V1 may help us address the inability of the adult brain to recover following a lesion.

**POSTERS-MON-080**

**LONG-TERM ANATOMICAL CHANGES TO THE VISUAL CORTEX FOLLOWING INJURY OF V1 IN EARLY AND ADULT LIFE**

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**Purpose:** Studies in the past have demonstrated that following a lesion of the primary visual cortex (V1) in early life allows for better recovery than an identical injury sustained in adulthood. However, the cellular / aural anatomical changes in the extrastriate cortex and visual thalamus associated with this phenomenon have yet to be characterised. **Methods:** Using the complex visual system of the marmoset monkey (Callithrix jaccus) as a modality, we examined changes in neonates and adults following partial unilateral ablation of V1. Ablations were performed in area V1 in the neonatal postnatal day (PD) 14 (n=2), and adult (>2 years, n=2) marmosets. Animals were allowed long-term recovery before undergoing transcendal perfusion for immunohistochemical analysis. Serial coronal 40µm sections were cresyl violet and myelin silver stained or immunolabelled with nonphosphorylated neurofilament (NFN), calsykalin (Cytb) and parvabumin (PV) to identify neuronal populations in areas V1, V2, V3, the middle temporal area (MT), the dorsal medial area (DM), and the dorsal lateral geniculate nucleus (dLGN). Volumes of areas were then quantified using Cavalieri’s method on Fiji. **Results:** Furthermore, the present study suggests a reduction in NFN average throughout all laminae in area MT in the lesioned hemisphere in adult lesioned animals yet MT in neonatal lesioned remains comparable to that of the control. An increase in density in Cb+ cells in layer 2 of the residual V1 compared to contralateral V1 can be observed in both cohorts as well as a volumetric decreases in the contralateral dLGN. **Conclusion:** Unilateral lesion to V1 has a significant affect on the thalamus and extrastriate areas of the visual cortex, in both hemispheres for both neonates and adult animals. Understanding the specific anatomical changes following a lesion of V1 may help us address the inability of the adult brain to recover following a lesion.
POS-MON-081
MARMOSET PRIMARY MOTOR CORTEX: CORTICAL INPUT TO DIFFERENT HISTOLOGICAL SUBDIVISIONS OF AREA 4
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We describe topographic and quantitative aspects of connections to the primary motor cortex (M1) in marmoset monkeys. In seven animals anaesthetised with Alfaxan (10mg/kg), ten retrograde tracer injections were placed in cytoarchitectural fields 4a, 4b and 4c, corresponding respectively to the representations of hindlimb/axial, forelimb, and head musculature in M1. Our data indicates that marmoset M1 receives substantial input from premotor area 6 (EM and 6DC), anterior parietal areas (3a, 3b and 1/2), posterior parietal area PE, and the secondary somatosensory area. Further input originates in posterior parietal areas PF/PFG and cingulate areas 23, 24 and 31. M1 has minor connections with area 6DR, and high-order visual and auditory association cortices. The cingulate cortex is most strongly connected with field 4a; whereas area 6DC, part of networks for reaching, is most strongly connected with field 4b. Field 4c has substantial connections with ventral premotor areas, and minor connections with prefrontal areas and gustatory association cortices. Field 4p is probably a component of neural circuits for coordinated actions involving reaching, grabbing and face/mouth movements such as eating. Conclusions: Marmoset M1 connections, including their topographic organisation, are similar to those reported for Old World monkeys. However, our results suggest some differences in cingulate cortex motor circuits compared with Old World monkeys, possibly reflecting a lack of precision grasping as well as an arboreal lifestyle reliant on leaping rapidly between branches. Otherwise, the somatomotor and visuomotor input from somatosensory and posterior parietal cortices suggest that these direct connections with M1 are established very early in primate evolution, and support the notion of functional heterogeneity within M1 involving multiple networks subserving somatomotor and visuomotor transformations.

POS-MON-082
SIMPLE SPIKE ACTIVITY OF CEREBELLAR PURKINJE CELLS ASSOCIATED WITH COMPLEX SPIKE WAVEFORM VARIATIONS
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Cerebellar Purkinje cells (PCs) produce two different types of action potentials: simple spikes (SSs) and complex spikes (CSs). SSs are comprised of a large initial spike, followed by a variable number of smaller secondary spikelets and such waveforms can vary. ‘Sporadic’ CSs have well defined initial and secondary spikelets while ‘non-sporadic’ CSs consist of the initial component and a collection of insubstantial ripples. There are conflicting reports regarding whether CS spikelet number is or is not related to SS rate. The aim of this study was to examine the interaction between CS waveform and SSs within the same PC and between pairs of functionally coupled cells. To this end, 42 PCs located in the A2 and C1 zones in crus II and the paramedian lobule were recorded in the ketamine/xylazine-anaesthetized rat. To estimate the correlation between the number of CS spikelets components and the rate of SSs, bootstrap analysis found that for 21/41 (51%) of PCs, a positive correlation was found between the number of CS spikelets and SS rate in the interval 500ms before the CS. A similar positive correlation was also observed when PC spike trains were modulated by peripheral simultaneous stimulation. Pairs of simultaneously recorded PCs that displayed synchronous CS activity, 9/15 (60%) had a significant relationship between SS rate and CS spikelets. In contrast, of 9 PC pairs that did not display synchrony, none showed such a relationship. The results provide evidence for an interaction between SS firing rates and subsequent CS waveform and may depend on whether a PC is coupled with another PC.

POS-MON-083
CORTICOSPINAL RESPONSES DURING DUAL MOTOR TASKING: PRELIMINARY FINDINGS USING TRANSCRANIAL MAGNETIC STIMULATION
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Purpose: Dual task (DT) research, using transcranial magnetic stimulation (TMS), has shown that when an individual performs two tasks concurrently, increased excitability and reduced inhibition are observed compared to that associated with single task (ST) performance. However, research to date has focused on corticospinal activity of fine motor DT and ST performance. The aim of this study was to use TMS to investigate DT performance during gross motor performance of the upper and lower limbs. Methods: Using a counterbalanced, cross-over design, healthy males (n=5; aged 32±10.6 years) completed, in randomized order, 4 conditions: ST easy (pincer grip of 15% of maximal voluntary contraction [MVC]±5%); ST difficult (15% MVC±1%); DT easy (pincer grip 15% MVC±1% during cycling at 10 revolutions per minute [rpm]); and DT difficult (pincer grip 15% MVC±1% during cycling at 10 rpm). TMS was delivered at 20% above active motor threshold. Twelve pulses were delivered, in sets of 3, spaced at random intervals of 5-8 s apart, with 1 min rest between each set and 2 mins between each condition. Results: Comparisons of grouped DT to ST conditions showed a 12.2% increase in MEP amplitude. The DT difficult condition showed a 27.2% increase in MEP amplitude compared to the ST easy (Cohen’s d 0.6) and 17.5% increase compared to the ST difficult (d 0.4) conditions. In addition, the DT difficult condition demonstrated a 5.9% and 4.5% decrease in silent period duration in comparison to the ST easy (d 0.4) and ST difficult condition (d 0.3) respectively. Conclusion: This preliminary study has shown changes in corticospinal excitability and inhibition during concurrent gross DT activity of the upper and lower limbs.

POS-MON-084
PROPERTIES OF CORTICOTHALAMIC PROJECTION NEURONS WITHIN LAYER 5 OF THE MOUSE MOTOR CORTEX
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The motor-associated corticothalamocortical (CTC) circuit synchronises neuronal firing within the primary motor cortex (M1). However, the phenotype of the layer V projection neurons within M1, that project to, and receive projections from, the thalamus is not known. Here we investigate whether thalamus-projecting neurons in the motor cortex constitute a discrete subtype within layer 5, and whether they receive thalamic projections. Injections of Alexa555-conjugated cholera-toxin B-subunit into the thalamus and contralateral M1 of young adult mice identified corticothalamic (CTH) and corticocortical (CC) neurons within layer 5 of slices of the motor cortex. Whole-cell electrophysiological recordings highlighted significant differences in the intrinsic electrical and firing properties of CTH and CC neurons (P < 0.05, t-tests). Post-hoc reconstruction of the recorded neurons CTH (n = 16) and CC (n = 15) also revealed key differences in their position within layer 5, their soma size, apical dendrite thickness, total dendritic length, tuft origin, tuft height, tuft width, and dendritic complexity (all P < 0.05, t-tests). To elucidate whether CTH neurons receive reciprocal projections from the thalamus we investigated the distribution of thalamic terminals within M1 using immunodetection of VGLUT2 (vesicular glutamate transporter 2), a marker for glutamatergic thalamic terminals. Interestingly, despite the change in size and shape of M1 across the rostro-caudal axis, the pattern of VGLUT2 labelling remained consistent. Furthermore, the dendrites of CTH and CC neurons exhibited different patterns of overlap with VGLUT2 expression (P < 0.05, two way ANOVA). Our results suggest that CTH neurons constitute a functionally and morphologically discrete subpopulation of M1 layer 5 projection neurons. Further investigations into the properties of these neurons may assist the development of therapeutic intervention for motor disorders.
CHARACTERIZING NEURONS OF THE MOUSE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA

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The study of the murine substantia nigra (SN) in models of Parkinson's disease has become increasingly important because of the availability of gene-targeted strains in the C57BL mouse. 

**Purpose:** We sought to characterize the dopamine neurons of the murine SN and ventral tegmental area (VTA) by studying the expression of biomarkers - antibodies that are dopamine-neuron specific, or detect calcium-binding proteins, as well as transcription and trophic factors. We wanted to determine if any biomarkers are associated with selective Parkinson's vulnerability represented in the dopamine neurons of SN compact part (A9) but not in the dopamine neurons of VTA (A10).

**Methods:** We studied neuronal morphology and biomarker distribution in the subregions of SN and VTA in the C57BL6/J mice (n=27). Results: C57BL6/J mice have five distinct subregions in SN. The reticular part has few dopamine neurons. The compact part is densely packed with dopamine neurons and is divisible into dorsal and ventral tiers as well as medial and lateral parts. The A9 contains a heterogeneous population of dopamine neurons. Compared with those of A10, dopamine neurons of A9 show constant expression of G-protein-regulated inward-rectifier potassium channel 2, but relatively low levels of tyrosine hydroxylase expression (p < 0.001). Compared with those of A10, dopamine neurons of A9 more often express pituitary homeobox 3 (a transcription factor) and deleted-in-colorectal cancer (a trophic factor), but express less often orthodenticle homeobox 2 (another transcription factor).

**Conclusion:** The different characteristics of dopamine neurons of SN and VTA might assist in deciphering the selective vulnerability of SN neurons to Parkinson's disease in humans.

LOCALISATION OF THE SPINAL MOTOR NEURONS INNERVATING THE HINDLIMB MUSCLES IN THE MOUSE

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**Purpose:** Our current purpose is to explore strategies for delivering therapeutic genes to specific segments of the motor neurons. This can be achieved by the intramuscular injections and retrograde transport of viral vectors containing the gene sequence of interest. Current research into amyotrophic lateral sclerosis/motor neuron disease focuses predominantly on the mouse hindlimb but relationship between the hindlimb muscles and the motor neurons that supply them has not been characterised in this species. Therefore, in this project, we aimed to characterise the muscle-motor neuron topography in the mouse hindlimb.

**Methods:** Mouse hindlimbs obtained through tissue sharing were dissected out, sectioned and analysed under epifluorescence for the localisation of the spinal motor neurons innervating the hindlimb muscles in naive C57BL6 mice that were anaesthetised with Isofluorane. One week post-surgery, the animals were intracardially perfused and the spinal cords dissected out, sectioned and analysed under epifluorescence for the presence of labelled motor neurons. 

**Results:** Motoneurons innervating the mouse hindlimb muscles are arranged in columns spanning several spinal segments. There is considerable overlap of the motor columns along all axes. 

**Conclusion:** The MEP and the motor column maps are instrumental in the selection of appropriate muscles for the delivery of therapeutic genes into specific segments of the spinal cord.

THE EFFECT OF MUSCLE FATIGUE ON THE STRETCH REFLEX AND JOINT STIFFNESS USING BROADBAND AND NARROWBAND PERTURBATIONS

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**Purpose:** This study investigated the effects of muscle fatigue on reflex behaviour and joint stiffness at the elbow joint in 22 subjects. Method: The elbow joint was rotated sinusoidally by signals with frequencies in the range of voluntary movement. Broadband (1-20 Hz) signals were delivered in one-minute epochs at rest and during a 15% background contraction. Narrowband (0.1, 0.5 and 1 Hz) signals were delivered to the joint at rest only. These measures were taken before and during a 30 minute recovery period which followed a 15 minute bicep-fatiguing protocol. The fatigue protocol consisted of 60 second maximal contractions separated by 30 second rest periods. Reflex data originated from biceps surface EMG and stiffness data from a torque transducer in series with the motor shaft. Results: During 15% contraction, mean reflex coherence and gain were decreased for 30 minutes after fatigue during application of the broadband signal. Mean torque stiffness, measured as the ratio of change in torque and change in joint angle, did not change after fatigue. For narrowband signals mean reflex coherence decreased for 0.1 and 0.5 but not for 1Hz. These changes did not reach statistical significance. This may have been partly due to variability in background contraction level between trials. Conclusion: Inconsistent responses to muscle fatigue were observed in 22 normal subjects using broadband and narrowband perturbations of the elbow joint. This suggests no systematic relation between muscle fatigue and stretch reflex response or joint stiffness when measured with continuous stretch perturbations.

SEXUAL DIMORPHISM IN COLONIC MIGRATING MOTOR COMPLEXES ON EXPOSURE TO CHOLERA TOXIN IS MEDIATE BY STEROID HORMONES

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**Purpose:** We previously demonstrated that cholera toxin (CT) significantly reduced the colonic migration motor complexes (CMMCs) in female C57Bl/6 mice. In the present study, the effect of luminal CT on CMMCs in colon isolated from female mice in two different stages of estrus cycle (proestrus with highest estrogen concentration and estrus with lowest estrogen concentration) was compared with age matched male C57Bl/6 mice. Methods: High resolution spatiotemporal maps of colonic motor patterns in vitro were constructed. Animals in the proestrus and estrus stages were selected after screening their vaginal smears. The colon was cannulated at each end and mounted horizontally in an organ bath containing physiological saline warmed to 37°C. The proximal end was connected to a reservoir of physiological saline, the distal end to an outflow tube. CT (1.25 μg/ml) was introduced to the lumen after control recordings with physiological saline and later washed out with physiological saline. Results: CMMCs recorded in colon isolated from females during estrus and male mice did not differ in control conditions (n > 6). However, CT produced a significant (p < 0.001) reduction in the frequency of CMMCs in female proestrus (60% of baseline). This no significant effect during proestrus or in male colon. The reduction occurred within 15 minutes of exposure to CT and was sustained for 1 hour, but reversed when CT was flushed from the colon lumen. Conclusions: Male colon and female mouse colon during proestrus behave similarly during exposure to CT. The significant reduction in colonic motility produced by CT during estrus may be due to the lower level of sex steroid hormones.
POS-MON-089  
THE ROLE OF PONTINE NUCLEI IN THE COORDINATION OF FICTIVE BREATHING AND SWALLOWING IN THE JUVENILE IN SITU RODENT PREPARATION  
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Purpose: During swallow, the inadvertent aspiration of ingested material is minimised by: 1) the temporary cessation of breathing movements, and 2) the activation of laryngeal adductor muscles that close the glottis and thus prevent entry to the lower airways. Pontine respiratory nuclei are critically involved in the cessation of inspiration during normal breathing, as well as providing pre-motor drive to laryngeal adductor motoneurons. Here, we investigated the involvement of the pons in the coordination of swallows with swallow-related changes in breathing. Methods: The in situ, perfused brainstem-spinal cord preparation of juvenile (P15-21) rat was used. Fictive breathing was monitored by recording phrenic, cervical vagus and laryngopharyngeal (L1) nerves. Fictive swallowing was monitored by recording the cervical vagus nerve. Results: Under control conditions. injection of distilled water (0.2ml) into the pharynx evoked successive swallows (typically 3-8), seen as brief (300-500ms), high amplitude bursts in cervical vagus nerve. Successive swallows were accompanied by a prolonged respiratory-related phrenic nerve discharge. Spontaneous swallows were rare. Pontine transections (5.5mm rostral to calamus scriptorius) that resulted in apneustic breathing were associated with an increased incidence of spontaneous swallows (n=8 rats). However, in most rats, the evoked swallowing pattern in response to water instillation into the pharynx remained similar to that under control conditions. Interestingly however, in n=3 rats, swallows were accompanied by large amplitude phrenic nerve “breakthroughs”. Conclusions: These preliminary results suggest a role of the pons in both the tonic inhibitory modulation of swallowing activity and the phasic, temporal coordination of swallowing with swallow apnoea. Dysfunction of the latter property may lead to aspiration.

POS-MON-090  
 EFFECT OF SINGLE VERSUS DUAL OREXIN RECEPTOR BLOCKADE ON THE CARDIOVASCULAR AND BEHAVIORAL RESPONSES TO NOVELTY STRESS IN RATS  
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Orexins (or hypocretins) play a critical role in the regulation of arousal. Orexins act via two receptors, OrxR1 and OrxR2, which have partly overlapping distributions in the brain. We have reported previously that blockade of OrxR1 and OrxR2 with the dual receptor antagonist Almorexant (Actelion Pharmaceuticals) reduces the cardiovascular and behavioral responses induced by novelty stress. The aim of the present study was to compare the effect of Almorexant to that of blockade of OrxR1 or OrxR2 individually with new selective antagonists. Rats implanted with telemetry probes were administered either with Almorexant, or the OrxR1 antagonist ACT-335827 (Actelion Pharmaceuticals), the OrxR2 antagonist EMPA (Roche) or vehicles, intraperitoneally or intragastrically, and after 2.5 hours were subjected to novelty stress for 30 minutes. In vehicle administered rats, novelty increased locomotor activity and evoked hypertension and tachycardia. When injected intraperitoneally, neither ACT-335827 (30 mg/kg, n=12) nor EMPA (30 mg/kg, n=8) had a significant effect on the locomotor or tachycardic responses induced by Novelty. However, intraperitoneal injection of Almorexant (30 mg/kg, n=7) significantly reduced the locomotor and hypertensive responses (65% and 54% reduction, respectively) although not the tachycardic response. When administered intragastrically, ACT-335827 (300 mg/kg, n=8) significantly reduced only the tachycardic response (46%), while Almorexant (300 mg/kg, n=10) significantly reduced all three responses (locomotor, 74%, hypertensive, 60% and tachycardic, 80%). The results indicate that blockade of one orexin receptor alone is not sufficient to produce the same effect as blockade of both receptors together at least in novelty stress. The two receptors may act synergistically.

POS-MON-091  
DO PROPRIOCEPTIVE AFFERENTS FROM THE NECK CONTRIBUTE TO THE VESTIBULAR MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY SUPPLYING THE LOWER LIMBS OF HUMANS?  
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Purpose: In order to maintain adequate blood flow to the brain during changes in posture muscle sympathetic nerve activity (MSNA) to the lower limbs is modulated by both the cardiovascular (baroreflex) and vestibular (vestibulospinal reflexive) systems. We have previously used sinusoidal galvanic vestibular stimulation to show that vestibular afferents produce a pronounced modulation of MSNA, although this is not as strong as that from the baroreceptors. Given that muscle spindle information from the neck influences the vestibular nuclei in the medulla, we tested the hypothesis that proprioceptive afferents from the neck also modulate MSNA. Methods: Four subjects lay supine on a specially designed table that fixed their head in space but allowed sinusoidal displacements of the body (35 degrees peak-to-peak displacement at 0.33-0.40 Hz) Results: Cross-correlation analysis revealed that MSNA was cyclically modulated during sinusoidal body-only angular displacements, (mean±SE 37±2±8%). Cardiac modulation at rest (75±1±7%) was reduced during body-only displacements (60±4±14%). Conclusion: These results suggest that proprioceptors in the neck, as well as vestibular and baroreceptor afferents, contribute to the physiological modulation of MSNA. This preliminary data suggests neck afferents can affect the cardiac modulation of MSNA, and that proprioceptors in the neck may contribute to the postural control of blood pressure.

POS-MON-092  
THE OXYGEN SENSITIVITY OF SYMPATHETIC PREMOTOR NEURONS DOES NOT INVOLVE HEME OXYGENASE 2 (HO-2)  
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Purpose: Sympathoexcitatory neurons within the rostral ventrolateral medulla (RVLM) are excited by hypoxia and are responsible for the increase in sympathetic nerve activity and blood pressure evoked by acute hypoxia. Previous studies implicate the enzyme HO-2 as a possible mediator of oxygen sensitivity in this region. However, the functional identity of neurons that express HO-2 is unknown. Our objective was to determine whether HO-2 is expressed in putative sympathetic premotor neurons and to determine whether its activation underlies their hypoxia sensitivity. Methods: We examined the distribution of HO-2 immunoreactivity in the brainstems of adult (n=3) and neonatal (n=1) rats in which spinally projecting neurons were labelled by cholera toxin B (CTB) previously injected into the thoracic spinal cord. We then examined the oxygen-sensitivity of bulbo spinal neurons (using the mitochondrial blocker NaCN as a hypoxia surrogate) in acute slices from p8-20 rats using whole cell recordings. Results: Although HO-2 was expressed within the RVLM region in all cases, immunoreactivity was concentrated in the facial nucleus and rostroventromedialmedulla, but absent in tyrosine hydroxylase and CTB immunoreactive neurons. NaCN caused reversible dose-dependent depolarizing currents in all neurons investigated that were much greater in bulbospinal neurons (n=20) compared to unlabelled neurons (n=18; 1113±412 vs. 1613±330 at 20mM NaCN; P<0.01). Responses were unchanged by perfusion with tetrodotoxin, suggesting that hypoxia sensitivity was intrinsic rather than synaptically mediated. In 5 hypoxia-sensitive bulbo spinal neurons filled with biocytin and subsequently counterstained for HO-2 locally, immunoreactivity was seen. Conclusion: These data support previous observations that sympathetic premotor neurons have an exaggerated response to hypoxia but suggest that HO-2 is not responsible for this effect.
**POS-MON-093**

**NEURAL CONTROL OF BLOOD FLOW TO CONTRACTING MUSCLE: CENTRAL COMMAND AND METABORECEPTORS**

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**Purpose:** There is a widespread view that sympathetic activity to contracting skeletal muscle increases as a function of intensity-related activation of the metaboreflex. However, this view is largely based on sympathetic nerve recordings to inactive muscle. We tested the hypothesis that muscle vasocostructor drive to contracting muscle decreases during contraction (functional sympatholysis) but increases during a period of post-exercise ischaemia due to the metaboreflex.

**Methods:** Muscle sympathetic nerve activity (MSNA) was recorded in 11 subjects via microneurography in the common peroneal nerve. Subjects performed 2-min static dorsiflexions of the ankle at 10% (n=11), 30% (n=11) and 50% (n=8) of maximal voluntary force, with and without muscle ischaemia, which was introduced in the second minute of contraction and continued 2-min post-exercise. Results: There was a significant effect of intensity on total MSNA (P<0.01), with increases from resting (mean ± SE) during the first minute of contraction of 141 ± 13% for the 10% contraction, 194 ± 32% for the 30% contraction and 468 ± 97% for the 50% contraction. Total MSNA during the second minute of contraction for the control and ischaemic conditions was significantly lower than the first minute (P<0.05), although remained significantly elevated above resting levels. In both conditions MSNA returned to baseline following contraction and this decrease persisted throughout ischaemia.

**Conclusions:** We conclude that the initial increase in MSNA to the contracting muscle is due to central command, which appears to be attenuated to both contracting and non-contracting muscles, and that the metaboreflex is not expressed in contracting muscle.

**POS-MON-094**

**EXAMINATION OF THE ANGIOTENSIN SYSTEM IN THE NUCLEUS OF THE SOLITARY TRACT IN MICE**

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**Purpose:** Viscerosensory neurons first synapse in the nucleus of the solitary tract (NTS) which is a key nucleus for the regulation of blood pressure (BP). Angiotensin II (AngII) acts in the NTS to modulate BP and this effect of AngII is concentration dependent, presumably due to the expression of its key receptor, the type 1A receptor (AT1R) on several different cell populations, including presynaptically on viscerosensory afferents and also on intrinsic neurons. These results were identified by AngII on the activity.

**Method:** The cellular distribution of the AT1R was examined in a transgenic mouse in which the AT1R promoter drives expression of green fluorescent protein (GFP). The mice were deeply anaesthetized, perfused with 4% paraformaldehyde and processed for double-labeling, fluorescence immunohistochemistry. Whole cell patch clamp recordings were made in NTS slices from tyrosine hydroxylase-GFP transgenic mice. AngII and AT1R antagonists were applied in superfusion. Results: Expression of GFP in the AT1R-promoter GFP mouse occurred almost exclusively in tyrosine hydroxylase-immunoreactive neurons of the A2 group. In the GFP positive neurons from the TH-GFP mouse AngII appeared to modulate resting membrane potential. Conclusion: The majority of AT1R expression on neurons intrinsic to the NTS occurs on catecholaminergic neurons. Application of AngII onto these neurons modulates their activity.

**POS-MON-095**

**EFFECTS OF ISCHEMIA AND REPERFUSION (I/R) ON THE P2X7 RECEPTOR AND ENTERIC NEURONS AFTER ADMINISTRATION OF BRILLIANT BLUE GREEN (BBG)**

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**Purpose:** Several studies have shown that injury can be attenuated by the antagonist of P2X7 receptor, Brilliant Blue Green (BBG). In the present work, we have analyzed the effects of the BBG on the P2X7 receptor and rat ileum myenteric plexus following I/R. Methods: The ileal artery was occluded for 45 minutes with an atraumatic vascular clamp. In the I/R 14 day group (n=5), BBG (50 and 100 mg/kg) or saline (vehicle, n=5) was given subcutaneous 1 hour after ischemia. In the I/R 24 h group (n=5), BBG was given once daily for the next 5 days. Also, we analyzed I/R 0 h group (n=5) (not reperfusion). Myenteric neurons were evaluated for immunoreactivity against the P2X7 receptor, nitric oxide synthase (NOS), neurofilament (NF) and choline acetyltransferase (ChAT). Results: P2X7 immunoreactivity against the P2X7 receptor, nitric oxide synthase (NOS), neurofilament (NF) and choline acetyltransferase (ChAT) was given subcutaneous 1 hour after ischemia. In the I/R 24 h group (n=5), BBG (50 and 100 mg/kg) or saline (vehicle, n=5)

**Discussion & Conclusion:** Lv-PRSx8-GFP expression predominantly occurred in astrocytes. The proportion of TH+ neurons infected by Lv-PRSx8-GFP was higher. Syn-GFP and Lv-PRSx8-GFP infected both TH- and TH+ NTS neurons. CBA-TdTOM, Lv-PRSx8-GFP and Lv-Syn-GFP predominantly infected visceral afferents and contains a heterogeneous population of neurons. Catecholaminergic neurons [tyrosine hydroxylase positive (TH+)] in the NTS are of interest because of their role in autonomic control of blood pressure. Aim: To optimise viral gene expression in different NTS cell types using different viral vectors. Methods: Adult wildtype mice were microinjected with viral vector (2x50nl). The NTS was located using stereotaxic coordinates. Viral vectors used: AAV-CBA-TdTom, LVR-Pax5-GFP, Lv-Syn-GFP, Lv-CAMKII-ChR2-GFP. Animals recovered for 2 weeks post-injection and were perfused with 4% PFA. The dissected brains were then post-fixed in PFA (4hrs) and sucrose (overnight). Free floating immunohistochemistry was performed on 40μm coronal sections to identify TH+ neurons and fluorescence expression. Results: Ad-CBA-TdTom, LVR-Pax5-GFP and Lv-Syn-GFP predominantly infected neuronal cells. Ad-CBA-TdTom infected TH+ neurons in the NTS. LVR-Syn-GFP and LVR-Pax5-GFP infected both TH- and TH+ NTS neurons. The proportion of TH+ neurons infected by LVR-Pax5-GFP was higher. Lvr-CAMKII-ChR2-GFP expression predominantly occurred in astrocytes. Discussion & Conclusion: The CBA promoter is reported to be a ubiquitous cellular promoter, whilst CAMKII and Synapsin promoter are considered to be pan-neuronal. We observed different transduction of cellular populations in the NTS, indicating the need to carefully examine cellular expression of each viral vector in specific regions. Stereotaxic injection of viral vectors enables preferential targeting of specific NTS cells. Of the 4 viral vectors, LVR-Pax5-GFP infected the highest proportion of catecholaminergic NTS cells.

**POS-MON-096**

**DIFFERENTIAL TRANSGENE EXPRESSION FOLLOWING STEREOTAXIC MICROINJECTION OF VIRAL VECTOR INTO THE NUCLEUS OF THE SOLITARY TRACT**

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**Background:** Stereotaxic microinjections of viral vectors are a powerful tool to manipulate protein expression in a cell- and region-specific manner. The specificity is produced by the viral coating and the promoter used to drive gene expression. The nucleus of the solitary tract (NTS) is located in the brainstem. It is the first site of termination for visceral afferents and contains a heterogeneous population of neurons. Catecholaminergic neurons [tyrosine hydroxylase positive (TH+)] in the NTS are of interest because of their role in autonomic control of blood pressure. Aim: To optimise viral gene expression in different NTS cell types using different viral vectors. Methods: Adult wildtype mice were microinjected with viral vector (2x50nl). The NTS was located using stereotaxic coordinates. Viral vectors used: AAV-CBA-TdTom, LVR-Pax5-GFP, Lv-Syn-GFP, Lv-CAMKII-ChR2-GFP. Animals recovered for 2 weeks post-injection and were perfused with 4% PFA. The dissected brains were then post-fixed in PFA (4hrs) and sucrose (overnight). Free floating immunohistochemistry was performed on 40μm coronal sections to identify TH+ neurons and fluorescence expression. Results: Ad-CBA-TdTom, LVR-Pax5-GFP and Lv-Syn-GFP predominantly infected neuronal cells. Ad-CBA-TdTom infected TH+ neurons in the NTS. LVR-Syn-GFP and LVR-Pax5-GFP infected both TH- and TH+ NTS neurons. The proportion of TH+ neurons infected by LVR-Pax5-GFP was higher. Lvr-CAMKII-ChR2-GFP expression predominantly occurred in astrocytes. Discussion & Conclusion: The CBA promoter is reported to be a ubiquitous cellular promoter, whilst CAMKII and Synapsin promoter are considered to be pan-neuronal. We observed different transduction of cellular populations in the NTS, indicating the need to carefully examine cellular expression of each viral vector in specific regions. Stereotaxic injection of viral vectors enables preferential targeting of specific NTS cells. Of the 4 viral vectors, LVR-Pax5-GFP infected the highest proportion of catecholaminergic NTS cells.
IS THERE PROPULSIVE MYOGENIC ACTIVITY IN THE DISTAL COLON? INVESTIGATIONS IN VITRO IN FOUR EXPERIMENTAL ANIMAL SPECIES

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Purpose: Intestinal motility is determined by myogenic and neurogenic mechanisms. Peristaltic propulsion in the distal colon is due to neurogenic mechanisms, but recent studies in rat distal colon suggested that after blocking neural peristalsis, propulsive myogenic mechanisms reappear after excitation with cholinergic agonists. We reinvestigated whether this was a species-specific effect. Methods: Motor activity of distal colon was studied from four adult mice, rats, guinea pigs and rabbits. Segments of entire (mice, rats) or isolated distal colon (guinea pigs, rabbits) were placed in an organ bath containing oxygenated Krebs solution (37°C). The preparations were distended by oral-end infusion of Krebs with the anal outlet blocked. Spatio-temporal maps of changes in diameter were constructed from video recordings. Results: Distension of distal colon in all four species elicited myogenic and neurogenic motor patterns. Aborally propagating peristaltic contractions were observed in all four species. These were blocked by tetrodotoxin (TTX; 0.6μM). The speed of propagation differed in all 4 species; mice (1.17±0.75 mm/s); rats (3.91±2.35 mm/s); guinea pigs (6.95±2.22 mm/s); and rabbits (8.57±2.69 mm/s). Addition of carbacol after TTX (0.1, 0.3 and 1μM) in mice and guinea pigs did not reproduce propagating contractions, while in rats and rabbits both anal and oral slowly propagating events (rats 1.66±0.91; rabbits 0.69±0.4 mm/s) were observed that did not resemble the pre-TTX treatment period. Conclusions: Enteric neural activity is essential for organised propulsive movements in the distal colon of all species studied, although underlying myogenic processes may be involved.

A COMPARISON OF RESPIRATORY AND CARDIAC MODULATION OF SYMPATHETIC NERVE ACTIVITY TO SKIN AND MUSCLE

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Purpose: It is well known that microelectrode recordings of skin sympathetic nerve activity (SSNA) in awake human subjects reveal spontaneous bursts of activity with no overt modulation by changes in blood pressure or respiration, in contrast to the clear cardiac and respiratory modulation of muscle sympathetic nerve activity (MSNA). However, cross-correlation analysis has revealed that the firing of individual cutaneous vasconstrictor neurones is temporally coupled to both the cardiac and respiratory rhythms during cold-induced cutaneous vasconstriction; the same is true of single sudomotor neurones during the heat-induced sweating (Macefield & Wallin, 1999). Here we used cross-correlation analysis to determine whether SSNA exhibits cardiac and respiratory modulation in thermoneutral conditions, and to compare modulation of SSNA with that of MSNA (Fatouleh & Macfieed, 2011). Methods: Oligounitary recordings of spontaneous SSNA were obtained from 21 subjects during quiet breathing. Respiration was recorded by a strain-gauge transducer around the chest and ECG recorded by surface electrodes. Respiratory and cardiac modulation of SSNA were quantified by fitting polynomials to the cross-correlation histograms constructed between the sympathetic spikes and respiration or ECG. Results: The amplitude of the respiratory modulation (51±2±4.4%) of SSNA was not significantly different from the amplitude of the cardiac modulation (46.7±2±3%). Both were comparable to the respiratory modulation of MSNA (49±5±6.0%), while cardiac modulation of MSNA was significantly higher (89±1±1.6%, p<0.001). Conclusions: We conclude that SSNA and MSNA share similar levels of respiratory modulation, the primary difference between the two being the marked cardiac modulation of MSNA provided by the baroreceptors.

DESCENDING MODULATION OF ABDOMINAL MOTOR OUTPUT BY PONTINE RESPIRATORY NUCLEI IN THE JUVENILE IN SITU RODENT PREPARATION

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Purpose: The iliohypogastric (abdominal; L1) nerve innervates the internal oblique muscles, which are strongly activated during forced expiration and expulsive respiratory-related reflexes (coughing and gagging). During normal breathing these muscles are largely quiescent, or express posture related activities. To date the parafacial/trerotropean nuclei and the nucleus retroambiguus are known to control the respiratory related abdominal motor outputs. Here, we investigated whether pontine respiratory-related nuclei may have a role in the control of abdominal motor output. Methods: Fictive breathing was monitored by recording phrenic (PNA), cervical vagus (cVNA) and abdominal nerves (AbNA) in an in situ perfused brainstem-spinal cord preparation of juvenile (P15-21) rat (n=6). Pontine transections were made 5.5mm rostral to calamus scriptorius. An arterial chemoreceptor reflex was triggered by bolus injections (0.1ml) of sodium cyanide (NaCN) before and after transection. Results: In all preparations AbNA exhibited low activity during the expiratory interval. Only NaCN transiently triggered pronounced activation of AbNA during late expiration. Rostrolateral pontine transections (parabrachial and Köller-Fuse nuclei removed; A5 intact) resulted in apneustic breathing patterns and absence of postinspiratory discharge in cVNA. Apneusis was associated with an increase in tonic AbNA nerve activity. After transection (n=4) large AbNA bursts emerged during expiration but these bursts did not oscillate consistently with inspiratory PNA. In n=2 preparations, only increase of tonic non-respiratory AbNA was observed. Moreover, chemoreflex elicited phasic recruitment of late expiratory AbNA was augmented (1.5x baseline) following pontine transection. Conclusions: The observed modulation of evoked or spontaneous AbNA after rostral pontine transection suggests that neurons of the parabrachial complex provide descending synaptic inputs that modulate strength and expression of abdominal expiratory motor activity.

A COMPARISON OF RESPIRATORY AND CARDIAC MODULATION OF SYMPATHETIC NERVE ACTIVITY TO SKIN AND MUSCLE

Fatouleh R.¹ and Macfieed V.¹,²
¹School of Medicine, University of Western Sydney. ²Neuroscience Research Australia, Sydney.

Purpose: It is well known that microelectrode recordings of skin sympathetic nerve activity (SSNA) in awake human subjects reveal spontaneous bursts of activity with no overt modulation by changes in blood pressure or respiration, in contrast to the clear cardiac and respiratory modulation of muscle sympathetic nerve activity (MSNA). However, cross-correlation analysis has revealed that the firing of individual cutaneous vasconstrictor neurones is temporally coupled to both the cardiac and respiratory rhythms during cold-induced cutaneous vasconstriction; the same is true of single sudomotor neurones during the heat-induced sweating (Macefield & Wallin, 1999). Here we used cross-correlation analysis to determine whether SSNA exhibits cardiac and respiratory modulation in thermoneutral conditions, and to compare modulation of SSNA with that of MSNA (Fatouleh & Macfieed, 2011). Methods: Oligounitary recordings of spontaneous SSNA were obtained from 21 subjects during quiet breathing. Respiration was recorded by a strain-gauge transducer around the chest and ECG recorded by surface electrodes. Respiratory and cardiac modulation of SSNA were quantified by fitting polynomials to the cross-correlation histograms constructed between the sympathetic spikes and respiration or ECG. Results: The amplitude of the respiratory modulation (51±2±4.4%) of SSNA was not significantly different from the amplitude of the cardiac modulation (46.7±2±3%). Both were comparable to the respiratory modulation of MSNA (49±5±6.0%), while cardiac modulation of MSNA was significantly higher (89±1±1.6%, p<0.001). Conclusions: We conclude that SSNA and MSNA share similar levels of respiratory modulation, the primary difference between the two being the marked cardiac modulation of MSNA provided by the baroreceptors.

ON THE MECHANISMS UNDERLYING PERIODIC BREATHING PATTERN IN NEONATES: THE ROLE OF POSTNATAL DEVELOPMENT AND NEUROCHEMICAL SYSTEMS

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Periodic breathing is common in premature infants and is also found in embryonic rats, and is characterized by periods of apnea interspersed amongst an otherwise regular breathing rhythm. This breathing pattern is rarely reported in adults, but is expressed in certain disease or conditions, e.g. Rett syndrome, Cheyne-Stokes breathing in heart failure, and at high altitude. Results: We discovered that periodic breathing pattern is common (>65%, n=25) in the in vitro pontomedullary-spinal cord preparation from rat pups on the day of birth (P0), but the occurrence of this breathing pattern declined with postnatal development. Chemical inhibition and physical removal of the pons eliminated the periodic breathing pattern at P0 (n=6). Interestingly, periodic breathing pattern could be restored in preparations from older rats (P2-P4, n=6-8) by decreasing the temperature from 27°C to 23°C, irrespective of the presence of the pons. In preparations held at 27°C, activation of GABA receptors (100μM GABA, n=5) significantly increased the periodicity of the breathing pattern (P<0.05), whereas bicuculline (10μM Bicuculline, n=5) changed the periodic breathing patterns to a regular, continuous rhythm (P<0.05). Intriguingly, antagonism of opioid receptors (naloxone, 1-5μM, n=6) did not affect the periodic breathing pattern. Conclusions: We have established an in vitro preparation that enables the study of the mechanisms underlying the generation of periodic breathing patterns in neonatal rodents. These data suggest the mechanisms involved in generating the periodic breathing pattern in neonates are modulated by postnatal development in the pons although mechanisms intrinsic to the medulla are also involved. Furthermore, these mechanisms are temperature sensitive and promoted by GABAergic neurotransmission.
POSTERS

**POS-MON-101**

**VPAC1 RECEPTORS EXPRESSED ON CHOLINERGIC SECRETOMOTOR NEURONS REGULATE SECRETION IN THE GUINEA-PIG JEJUNUM**

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**Purpose:** There are two main classes of secretomotor neurons in the enteric nervous system: cholinergic (contains choline acetyltransferase, ChAT and neuropeptide Y, NPY) and non-cholinergic (contains vasoactive intestinal peptide, VIP). VIP is a potent secretagogue implicated in pathophysiological conditions and may be a putative neurotransmitter between VIP neurons. However, it is still unclear whether VIP has neural actions involved in secretion and if so, which receptor subtypes (VPAC1 and VPAC2) are expressed by enteric neurons. We examined the role of VPAC1 receptors (VPAC1-R) in chloride secretion. **Methods:** Guinea-pig jejunal mucosal-smucumucosal preparations were mounted in Ussing chambers to measure short circuit current (Isc) and hence chloride secretion. Drugs were added to the serosal half-chamber. Proportions of submucosal neurons (marked by pan-neuronal marker HU) expressing ChAT, NPY, and VPAC1-R were examined immunohistochemically. **Results:** VP (50 nM) evoked an increase in Isc (n=5). Tetrodotoxin (1 µM) abolished the VP response but did not affect the VIP response by 21% (n=4; P<0.05). PG97-269 (1 µM; VPAC2-R antagonist) attenuated the VP response by 62% (n=5; P<0.05) and the residual response was unaffected by tetrodotoxin. Thus, there was a small neuronal component of VIP-mediated secretion involving VPAC1-R. Mucarinc receptor blockade with hyoscine (10 µM) reduced the VIP response by 30% (n=5; P<0.01), suggesting involvement of cholinergic neurons. The cell bodies and axons of neurons found in the periphery of submucosal ganglia expressed VPAC1-R. 82% of ChAT-positive neurons and 87% of NPY-positive neurons also expressed VPAC1-R. **Conclusion:** This is the first demonstration that VIP-induced chloride secretion involves activation of VPAC1 receptors on cholinergic submucosal neurons.

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**POS-MON-103**

**CATECHOLAMINES IN THE MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS ALTER CARDIORESPIRATORY AND METABOLIC FUNCTIONS**

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**Purpose:** Methamphetamine (MET) blocks and reverses monoamine transporters and its administration evokes behavioural changes associated with frontal brain regions. MET also evokes autonomic effects. We have shown that disinhibition of the medial prefrontal cortex (mPFC) changes cardiorespiratory and metabolic function. We sought to determine whether alterations in monoamine concentrations in the mPFC and nucleus accumbens (NAC) mediate changes in cardiorespiratory and metabolic function. **Methods:** Electrophysiological experiments were performed in urethane-anaesthetised, artificially ventilated, vagotomised, male Sprague-Dawley rats. Microinjections of METH (5,15,30 µg/kg), dopamine hydrochloride (DA 3.10,30,90 µg/kg) or L-norepinephrine (NA 3.10,30 µg/kg) were made into the infralimbic cortex of the mPFC and NAC. Changes were recorded in IBAT, heart rate (HR), expired CO2, phrenic nerve amplitude and frequency (PNamp and PNf), mean arterial pressure (MAP), splanchnic and lumbar sympathetic nerve activity. **Results:** MET in PFC evoked respiratory depression at low doses but increased all parameters at high doses. DA evoked increases in thermogenic and metabolic outflows (IBAT;2.2±0.3°C, HR;21±4bpm and expired CO2;0.6±0.15% (n=5) and in respiratory frequency (PN1;11±1bpm, and, PNamp;13±15% (n=5) with little change in other parameters only at the highest dose with only respiratory depression evoked at low doses. In contrast in the PFC NA evoked dose dependent increases in MAP (18±2mmHg, 100µg/kg), IBAT (0.8±0.15°C), HR (16±2bpm) and expired CO2 (0.35±0.07%) and respiratory function. **Conclusions:** In PFC respiratory depressive effects were evoked by low doses of MET and these were mimicked by low doses of DA. The increased cardiovascular, respiratory and metabolic function evoked by MET in PFC was mimicked by NA, although DA may also contribute. Alteration in monoamine levels in frontal brain regions alters cardiorespiratory and metabolic function.

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**POS-MON-102**

**DIFFUSION-LIMITED KINETICS OF PEPTIDE BINDING TO G-PROTEIN COUPLED RECEPTOR-ENRICHED DOMAINS ON INTACT SYMPATHETIC NEURONS: HIGH-SPEED QUANTITATIVE IMAGING AND FLUORESCENCE CORRELATION SPECTROSCOPY**

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**Purpose:** We showed previously that AT1 receptors (AT1R) for angiotensin II (AngII) expressed by native sympathetic neurons in guinea-pig coeliac ganglion exhibit little functional desensitisation. Thus, they continue signalling as long as supra-threshold concentrations of AngII exist. Conventional pharmacokinetics assume that interactions between free agonist and receptors are not limited by diffusion. However, this may not be true in native tissue. Therefore we used high-speed photon counting confocal microscopy and fluorescence correlation spectroscopy (FCS) to measure directly diffusion of AngII labelled with AlexaFluor dyes in the extracellular space surrounding coeliac ganglion neurons. **Methods:** We used a Leica SP5 confocal microscope with avalanche photodiodes and resonant scanner enabling imaging at 20 frames/s with a single pixel capture time of 0.5µs. Diffusion of AngII-Alexa647 was measured using FCS at multiple sites and time points in acutely isolated coeliac ganglia (n = 12). Control data were obtained from CHO cells transfected with eGFP. Photon counting data were analysed with ISS-Vista, SPSS and Excel. Images were analysed with ImageJ. **Results:** AngII-A647 (1.10mM) binding to CHO cells expressing AT1R-eGFP showed conventional binding kinetics. However, comparable binding data in native coeliac ganglion neurons could not be fitted with conventional pharmacokinetic equations, mainly due to significant retention of peptide in the extracellular space. FCS showed that the diffusion coefficient of AngII-A647 in this area was up to two orders of magnitude slower than that in free solution. **Conclusion:** Extra-cellular connective tissue severely limits AngII diffusion leading to non-conventional pharmacokinetics that facilitate long-term signalling in sympathetic neurons.
POS-MON-105

SELECTIVE AT1A RECEPTOR KNOCKOUT FROM CATECHOLAMINERGIC NEURONS REDUCES THE HYPERTENSION INDUCED BY CHRONIC LOW DOSE INFUSION OF ANGIOTENSIN II

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Purpose: Chronic low dose systemic infusion of angiotensin II (Ang II) induces hypertension via activation of the Ang II type 1A receptor (AT1-R). Whilst renal expression of this receptor is important for the hypertension, other sites appear to be involved. We tested the hypothesis that AT1-Rs on catecholaminergic neurons are required for Ang II-induced hypertension. Methods: Mice with cell-specific deletion of AT1-R from catecholaminergic neurons were generated by crossing AT1-R floxed mice with mice expressing cre-recombinase (Cre) under the control of the tyrosine hydroxylase (TH) promoter. AT1-R floxed (AT1-R+/−TH-Cre−/−) and AT1-R+/−TH-Cre−/− (Control) mice were used for this study. Ang II (500ng/kg/minute) was infused subcutaneously via an osmotic minipump for 3 weeks. Telemetry devices or a noninvasive tail cuff system were used to record BP and heart rate (HR) and autonomic function assessed. Dihydroethidium (DHE) was used to measure superoxide formation in the brain. Results: Baseline systolic BP was not different between the groups (109±1mmHg vs. 108±2 mmHg). Infusion of Ang II induced a gradual pressor response that was greater in control mice than CAT-KO mice (148±4mmHg vs. 123±4 mmHg at day 13). Sympathetic activation induced by Ang II was reduced in CAT-KO mice. The Ang II-induced increase in superoxide production in the brain was only reduced in the rostral ventrolateral medulla of CAT-KO mice. Conclusions: These data demonstrate that AT1-R expression by catecholaminergic neurons is required for full development of angiotensin-dependent hypertension.

POS-MON-106

[125I]-GALANIN RECEPTOR BINDING IN THE BRAIN OF ALCOHOL-PREFERRING AND NON-PREFERRING RATS

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Purpose: We have previously shown that treatment with the galanin-3 receptor (GALR3) antagonist, SNAP 37889, reduced fixed-ratio operant responding for ethanol, sucrose and saccharin solutions in the alcohol-prefering (P) rat. Alcohol has a high energy content so the mechanisms that modulate alcohol consumption are speculated to include hypothalamic inputs that also act to regulate food intake. The present study therefore aimed to investigate changes in galanin receptor binding in brain regions known to be involved in addiction and feeding. Methods: P rats (n=4/5 per group) were given continual access to 5% (v/v) ethanol, 5% (w/v) sucrose, 0.1% (w/v) saccharin or water, along with a second bottle containing water as part of a two-bottle free choice paradigm. An additional group (n=5) of non-prefering (NP) rats were given access to two bottles of water, to later assess differences in galanin receptor binding between the P and NP strains. A second cohort of P rats were divided in to four treatment groups (n=5) to receive either a single injection or daily injections for 10 consecutive days of vehicle (5% DMSO and 1% HMC in saline; 1 ml/kg) or SNAP 37889 (30 mg/kg, i.p.). Brain tissue was processed for galanin receptor autoradiography and the level of [125I]-galanin binding quantitatively analysed in selected brain regions. Results: Galanin receptor density was found to be significantly lower in the nucleus accumbens (NAc) in P rats, when compared with NP rats (p<0.0001). 10 days of treatment with the GALR3 antagonist, SNAP 37889, induced a significant increase in galanin binding in the NAc (p<0.001). Conclusion: These data suggest a role for galanin at the level of the NAc, which is speculated to contribute functionally to the intake of alcohol.

POS-MON-107

THE EARLY LIFE NUTRITIONAL ENVIRONMENT ACUTELY ALTERS THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS FUNCTION

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The early life nutritional environment can permanently modify the hypothalamic-pituitary-adrenal (HPA) axis. We have previously seen these effects are sex-dependent, thus, female rats made overweight by neonatal over-feeding elevated basal corticosterone in males but had no effect in females and there was no effect on stress hormone response. These changes would be sex-dependent. We used corticosterone assays to detect changes in the HPA axis function. At this age neonatal over-feeding elevated basal corticosterone in males but had no effect in females and there was no effect on stress hormone levels of neonatal under-feeding. These results indicate neonatal dietary manipulations can alter glucocorticoid profiles, potentially affecting HPA axis function long-term.

POS-MON-108

CRF1 RECEPTORS IN THE VENTRAL TEGMENTAL AREA REGULATE COCAINE-SEEKING IN MICE

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Purpose: Stress is associated with drug addiction, contributing to drug use and propensity to relapse. Corticotropin-releasing factor (CRF) has been implicated in this relationship, and the ventral tegmental area (VTA) is a suggested locus for this action. However, whether CRF within the VTA has a role in drug-seeking in the absence of acute stressors, and the receptor subtype(s) mediating this effect remain unanswered. Therefore, we assessed the effects of CRF receptor type 1 (CRF1) signaling within the VTA on cocaine and sucrose-seeking behaviour in mice using RNA interference. Methods: Mice were stereotaxically injected with lentivirus encoding short hairpin RNAs (shCRF1R1 or scrambled control) targeting the VTA. The effects of this treatment were examined using an operant self-administration paradigm for cocaine (shCRF1R1 virus n=27, control virus n=10) and sucrose (shCRF1R1 virus n=20, control virus n=9). We then examined cue-induced reward seeking after a period of enforced abstinence. Results: Intra-VTA infusions of lentiviral-shCRF1R1 had no effect on the acquisition and maintenance of self-administration of either cocaine or sucrose. However, cue-induced cocaine-seeking following abstinence was markedly attenuated in shCRF1R1 treated mice compared to those receiving the control virus (p<0.01). In contrast, sucrose-seeking as not affected by intra-VTA lentivirus. Conclusion: Our data suggest that CRF1 in the VTA are critical for cue-induced cocaine seeking, but not for sucrose-seeking, suggesting the role of CRF1 may be specific for drugs of abuse rather than rewards in general. Furthermore, it appears that CRF1 in the VTA have a role in relapse behaviours beyond conditions that focus on stress.
CENTRAL ADMINISTRATIONS OF PALMITIC ACID AND ARACHIDONIC ACID DECREASE CENTRAL LEPTIN SENSITIVITY IN MICE

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Purpose: Leptin inhibits feeding and increases energy expenditure through the central nervous system. High-fat diet with saturated fatty acids (SFA) or n-6 polyunsaturated fatty acids (n-6 PUFA) has been reported to induce central leptin resistance and obesity. However, little is known if central administration of SFA or n-6 PUFA can reduce central leptin sensitivity. This study examined the central leptin sensitivity in response to intracerebroventricular (i.c.v.) injection of SFA, palmitic acid (PA) and n-6 PUFA, arachidonic acid (ARA) in mice. Methods: After overnight fasting, C57Bl/6J male mice (n=24/group) were i.c.v. injected with either PA (50μmol/2μl), ARA (50μmol/2μl) or vehicle (saline, 2μl) prior to i.c.v. injection of leptin (0.5μg/2μl) or saline followed by refedding for 24 hours ad libitum on normal diet. Food intake was measured at 1, 4, 16 and 24 hours after leptin i.c.v. injection. Body weight gain was measured 24 hours after leptin injection. Results: PA and ARA i.c.v injection did not significantly change food intake in mice compared with vehicle group. In vehicle group, central leptin administration significantly suppressed food intake at 1hour (64.0%, p<0.001), 4-hour (54.98%, p=0.003), 16-hour (54.34%, p<0.001) and 24-hour (52.72%, p<0.001). In PA and ARA pre-treatment group, leptin did not significantly suppress food intake compared with saline injection at 16 and 24 hour. Furthermore, leptin significantly decreased body weight gain for 24 hours in vehicle group (404.69%, p<0.001). However, in ARA pre-treatment group, there was no significant difference in the body weight gain after central leptin and saline injection. Conclusion: This study suggested that central administration of saturated fatty acid PA and n-6 unsaturated fatty acid ARA could induce central leptin resistance.

EFFECT OF WESTERN DIET ON BRAIN C-FOS IMMUNOREACTIVITY IN RATS

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Purpose: Recent evidence shows an association between obesity and cognitive decline. The present study aimed to determine whether consumption of a western diet (WD) produces changes in neuronal activation in brain regions involved in memory regulation in rats. Methods: Two groups of male Long Evans rats were fed either normal chow (Con) or WD (23% fat, 0.19% cholesterol) for 15 weeks (n=12 per group). Body weight and food intake were measured twice weekly. c-Fos immunohistochemistry was carried out in specific areas of hippocampus (CA1, CA2&3 and dentate gyrus), retrosplenial cortex (Rbg and Rdg), prefrontal cortex (infralimbic, prelimbic and anterior cingulate regions), nucleus accumbens, paraventricular nucleus of thalamus (PVT) and paraventricular nucleus and arcuate nucleus of hypothalamus. Results: Analysis showed that consumption of a WD significantly decreased basal c-fos immunoreactivity in the CA2&3 regions of hippocampus, PVT and prelimbic region. Conversely, c-fos immunoreactivity was increased in retrosplenial cortex. No significant changes were observed in CA1, dentate gyrus, nucleus accumbens, infralimbic and cingulate cortices and regions of the hypothalamus after consumption of a WD. Conclusion: These results demonstrate that consumption of a western diet for 15 weeks affects neuronal activation in specific brain regions involved in memory regulation.

CENTRAL ADMINISTRATIONS OF PALMITIC ACID AND ARACHIDONIC ACID DECREASE CENTRAL LEPTIN SENSITIVITY IN MICE

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Purpose: Neuroinflammation is relevant to a number of neurodegenerative diseases like Parkinson's disease. Interestingly, severe inflammation in the substantia nigra (SN) accelerates the onset and progression of Parkinson's disease. In particular, the neutrophil infiltration in SN has a determinant effect on dopaminergic neuronal death. PXS-4681A is a potent and selective inhibitor of the semicarbazide-sensitive amine oxidase (SSAO) also called vascular adhesion protein-1 (VAP-1) which is known to reduce inflammation-driven neutrophil influx in various tissues. PXS-4681A is anti-inflammatory and reduces oxidative stress produced by the catalytic function of SSAO/VAP-1. To study the effects of PXS-4681A on brain inflammation we stimulated leukocyte recruitment through systemic administration of lipopolysaccharide (LPS, E.coli) and measured neutrophil recruitment, microglia and astrocyte activation. Methods: Long Evans rats (n=15) received 3 i.p. injections of LPS or PBS (10mg/kg at 0h, 3mg/kg at 6h, 1mg/kg at 24h). One group received PXS-4681A i.p. 2mg/kg at 0 and 24h, while the other two groups received PBS. Immunofluorescence staining for myeloperoxidase and rat endothelial cell (RECA-1) was used to quantify the neutrophils. Microglia was revealed by iba1 antibody and was quantified as cell number per area while morphological changes were analysed with Neuroulida software. The astrocyte response was evaluated using western blot for glial fibrillary acidic protein. Results: SSAO/VAP-1 seems to contribute to LPS-induced inflammation in brain and preliminary data suggest that PXS-4681A significantly diminishes microglia response in the SN and in the dorsolateral striatum. PXS-4681A also decreased neutrophil extravasation and astrocyte activation in these areas. Conclusions: PXS-4681A showed a clear anti-inflammatory effect after systemic LPS. The results suggest that SSAO/VAP-1 inhibitors could be beneficial in the treatment of neurodegenerative diseases.

METHODOLOGICAL APPROACHES TO QUANTIFICATION OF MICROGLIAL-SYNAPTIC CONTACTS

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Purpose: Recent insights into microglial function have revealed a dynamic role for microglia at synaptic junctions in the healthy brain. Microglia have been implicated in the support and renewal of synaptic structures in response to environmental inputs. Here, we present a variety of techniques for imaging microglia and synapses using iontophoretic dye filling of neurons, direct application of fluorescent lipophilic dyes, and Diolistic labelling of neurons combined with immunofluorescence to quantify the extent of physical contact in the rodent brain. Methods: Rodent tissue was fixed with acrolein or paraformaldehyde and sectioned at 100 μm on a vibratome. Brightfield visualisation of neuronal soma enabled somal penetration with glass micropipettes filled with fluorescently labelled lipophilic dyes and Diolistic labelling of neurons combined with immunofluorescence to quantify the extent of physical contact in the rodent brain. Results: Dye filling was performed using dyes suitable for identification of specific regions, and the extent of physical contact was validated using immunofluorescent labelling of neurons. Conclusions: These results demonstrate that consumption of a western diet for 15 weeks affects neuronal activation in specific brain regions involved in memory regulation.

METHODS OF ANALYSIS TO QUANTIFY MICROGLIAL-SYNAPTIC CONTACTS

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**POST-MON-113**

**POST-TRANSLATIONAL CLEAVAGE OF THE MRF PROTEIN YIELDS A FUNCTIONAL TRANSCRIPTION FACTOR**

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**Purpose** Myelin gene regulatory factor (MRF) has an essential role in the myelination of the central nervous system via inducing the transcription of myelin genes. However, the molecular mechanisms by which it does so remain unclear, and it is predicted to bind both a DNA-binding and a transmembrane domain, raising questions of whether it would be expected to be localized to the nucleus. Here, we study the processing, localization and functioning of the MRF protein.

**Methods** We performed in silico analysis of the MRF amino acid sequence to identify conserved functional domains and motifs, PCR site directed mutagenesis, immunocytochemistry and western blots were used to study the molecular mechanisms of MRF. **Results** Western blot analysis showed that besides its full-length form, MRF is present in cells as truncated N and C-termini. Furthermore, immunocytochemistry showed that while the N-terminal of MRF localizes to the nucleus, the C-terminal remains extracellular. In silico analysis of the MRF protein suggested that besides the putative DBD and TMD, MRF is predicted to contain a nuclear localization signal (NLS) in its N-terminal region. Indeed, site directed mutagenesis of this predicted NLS prevented the N-terminal from entering the nucleus of cells of an immortalized oligodendrocyte precursor cell line (n=2 independent experiments). **Conclusions:** These results are consistent with MRF undergoing processing that separates its N and C termini, with the N-terminal subsequently acting as a transcription factor that directly regulates the expression of myelin genes.

**POST-MON-114**

**PROTEOMIC ANALYSIS OF PRIMARY ASTROCYTES CARRYING MUTANT TDP43**

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**Purpose:** The pathological signature of several neurodegenerative diseases including motor neuron disease (MND) is accumulation of ubiquitinated cytosolic protein aggregates that include the nuclear TAR-DNA binding protein TDP-43. This protein regulates transcription, transport and post-transcriptional modification of mRNAs. TDP-43 pathology is triggered by abnormal processing and cytosolic aggregation of the protein which is promoted by mutations in the TDP-43 gene and is associated with nuclear depletion of the protein. Mutant TDP-43 causes familial forms of MND, MND-like disease in animals and kills motor neurons in culture. TDP-43 pathology is also found in astrocytes, cells that are critical determinants of non-cell autonomous injury and disease progression in MND; whether astrocytes are functionally affected in TDP-43 proteinopathies is presently unknown.

In this study we have investigated effects on protein expression in astrocytes transfected with mutant TDP-43. **Methods:** Primary mouse cortical astrocytes were transfected using nucleofection with TDP-43 mutations Q133k and A315T and compared to cells transfected with a control plasmid. Soluble proteins were extracted from cytosolic and nuclear fractions and 2D-electrophoresis and DIGE were performed using 24cm pi pI3-11 NL strips. Gels were analysed using Deyder 7.0. Results: Initial analysis of 2D-gels identified 110 differentially expressed proteins (>3 fold) in astrocytes carrying the mutant TDP43 compared with controls (n=3). A smaller number of proteins also exhibited a gel shift consistent with changes in phosphorylation. Preliminary DIGE experiments further identified 60 decreased and 7 increased (>3 fold) proteins whereof 52 proteins changed more than 5-fold. Western blot analyses confirmed changes in PS3 and GLT-1 (n=3) expression. Mass spectrometry analyses are ongoing and results of these will be available in the final presentation. **Conclusion:** This is the first study demonstrating global effects on protein expression in TDP-43 proteinopathies. These data will prove invaluable for identification of new pharmacological approaches for treatment of these and related conditions.

**POST-MON-115**

**THE INFLUENCE OF TRKB SIGNALLING ON OLIGODENDROCYTE MYELINATION**

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**BDNF** is known to promote central nervous system (CNS) myelination both in vitro and in vivo. We have identified that BDNF acts through oligodendroglial TrkB receptors to promote myelination in vitro. **Purpose:** Here we investigate a) the influence TrkB exerts on myelination in vivo, and b) the pathways downstream of TrkB which promote myelination in vitro. **Methods and Results:** Mice with a targeted deletion of TrkB in the spinal cord (TrkB [fl/fl]) and b) the pathways downstream of TrkB which promote myelination of oligodendroglial cells in the spinal cord revealed no significant differences to control mice during postnatal development (n=5 per time point). Our data have shown that TrkB activation correlates with MAPK/Erk pathway activation and myelination in vitro. Here we show that in OPCs infected to overexpress Erk1 or Erk2, Erk2 is the key MAPK enhancing myelination (n=3). Erk2 is known to exert some of its effects through direct transcription factor phosphorylation. Our in silico analysis identified potential Erk2 binding domains and phosphorylation sites in several oligodendrocyte-specific transcription factors required for myelination. Co-immunoprecipitation experiments show an interaction between Erk1/2 and these transcription factors occurs in vitro (n=3). Investigations into the nature of these interactions and into their functional consequences are ongoing. **Conclusion:** Collectively, this work suggests a novel role for Erk2 signalling within oligodendrocytes that regulates CNS myelination, possibly via activation of oligodendroglial transcription factors. As Erk1/2 is activated by multiple receptors, these data also suggest that in the absence of TrkB activation, other receptors can compensate resulting in a normally myelinated CNS in vivo.

**POST-MON-116**

**ROLE OF GPR62 IN CENTRAL NERVOUS SYSTEM MYELINATION**

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**Purpose:** Current myelination research in central nervous system (CNS) myelination has focused on factors affecting oligodendroglial differentiation, yet the molecular interactions that occur post-differentiation between that oligodendrocyte and axon during developmental myelination are still unclear. Recently, the transmembrane protein G-protein coupled receptor gpr62 was identified as an oligodendrocyte specific protein specifically expressed by myelinated oligodendrocytes during development, indicating its potential role in the glial-axonal interaction during myelination. **Methods:** Here we generated a mouse strain with a germ-line knockout of gpr62 to investigate if it has a role in myelination or axoglial interactions. Electron microscopy and immunohistochemistry were performed on the optic nerve, spinal cord, and corpus callosum of knockout and wildtype mice at 3 weeks, 8 weeks, and 26 weeks of age (n=3 genotype/time-point). Quantitative reverse-transcriptase PCR analysis of transcripts for myelin genes in hemi-brain lysates was performed to determine the effect of Gpr62 on gene transcription. **Results:** Detailed electron microscopy in all three regions of the brain showed no significant difference in the number of axons/myelinated, indicating a dispensable role in initiating myelination. Similarly, immunohistochemical analysis of the oligodendrocyte cell lineage showed no significant changes in cell densities of the oligodendrocyte precursor cells or mature oligodendrocytes, likely precluding Gpr62 as a regulator of oligodendrocyte differentiation. Interestingly, upon quantitative reverse-transcriptase PCR analysis of transcripts for myelin genes, we observed a significant increase in mrf transcripts for myelin genes after postnatal myelination. Further analysis to fully investigate if it has a role in myelination or axoglial interactions. **Conclusion:** Taken together, these results implicate Gpr62 as a potential dopaminergic factor of plp1 expression after postnatal myelination. Further analysis to fully characterize the role of Gpr62 is fundamental to potentially discover a novel axonal-glial signalling pathway.

**POSTERS**

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A QUANTITATIVE STEREOELEOMETRIC AND MORPHOLOGICAL ANALYSIS OF MICROGLOIA IN THE PREFRONTAL CORTEX

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Purpose: Several recent studies have indicated that microglia, particularly within the prefrontal cortex, are disturbed by exposure to chronic psychological stress. Morphological alterations in these microglia suggest a functional change, which is intimately linked to cell structure. However, almost nothing is known from a neuroanatomical perspective about microglia within the normal prefrontal cortex. Accordingly, in the current study we set out to determine the absolute numbers of microglia within the healthy prefrontal cortex, and examined whether there were any differences in microglial density across each of the five cortical layers, followed by a systematic survey of microglial morphology.

Methods: High resolution images of cells labelled with the microglia-specific ionized calcium binding adaptor molecule 1 were taken on a Zeiss Axioplan photomicroscope. Both unbiased stereological counting and exhaustive sampling (n=250) were used to provide quantitative morphological measures, followed by Sholl analysis to determine morphological features as a function of distance from the soma. Results: Our results indicate that while there are moderate fluctuations in the laminar density of microglia, the cells are effectively homogeneously distributed. Regarding the cells' morphology, we discovered that microglia vary enormously in size, and can clearly be subcategorized on the basis of the area that they occupy within the cortex, with small and large cells having quite distinct morphological characteristics. Conclusion: To our knowledge this is the first ever study to systematically characterise laminar density and morphology of microglia within the prefrontal cortex.

SUPPRESSION OF LPS-INDUCED MICROGLIA ACTIVATION BY SVNNP THROUGH MODULATING NUCLEAR FACTOR KAPPA B AND JNK SIGNALING CASCADES

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Purpose: Scorpion has been used in traditional Chinese herbal formulas to treat neurodegenerative disorders for a long time in the Orient, but with its mechanisms not completely clarified. Scorpion venom neuro-nourishing peptide (svNNP), an active component of this herb, is extracted from Buthus martensi Karsch (BmK) by means of a special method (National Invention Patent No: ZL01 1 06166.92, China). Our experiments showed that svNNP has neuroprotective effects. In this study, we explored the mechanisms of neuroprotection by svNNP, particularly its anti-inflammatory effects in microglia.

Methods: Lipopolysaccharide (LPS) was used to induce an inflammatory response in cultured primary microglia or murine BV-2 microglial cells(n=3-5). Nitric oxide (NO) production was determined using the Griess Reagent. The levels of tumor necrosis factor (TNF-α) was measured using ELISA kits. Signaling molecules were analyzed by western blotting, and activation of nuclear factor κB (NF-κB) was measured by ELISA and Immunocytochemistry. Results: We observed that svNNP reduced LPS-induced production of NO and TNF-α, and inhibit expression of inducible NO synthase (iNOS) and TNF-α in rat primary microglia or BV-2 cells. LPS-induced nuclear translocation and DNA binding activity of NF-κB, as well as c-Jun N-terminal kinase (JNK) and p38 phosphorylation were repressed by svNNP. Conclusion: Together, the present study reported the anti-inflammatory activity of svNNP in microglial cells and suggested that svNNP might have therapeutic potential for microglia-mediated neuroinflammation, thus serve as a new candidate drug for neurodegenerative diseases.
POS-MON-121

BEHAVIORAL REGULATION OF DOPAMINE NEURONS IN ADULT MOUSE SUBSTANIA NIGRA

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Purpose: Failure of acquisition and maintenance of the dopamine (DA) phenotype by cells in the adult substantia nigra pars compacta (SNc) is a major problem confronting cell-replacement therapies for Parkinson's disease. We recently demonstrated that the number of SNc DA neurons is altered following infusion of ion-channel drugs into SNc, indicating that their DA phenotype is activity-dependent. This led to the hypothesis that the number of SNc DA neurons is regulated during behavior.

Methods: To test this adult (≥8-week old) mice were subjected to two behavioral protocols: (1) mating, where a male and female mouse were housed together for 10 days (controls were male-male and female-female pairs, n=4/group); and (2) environment enrichment, where male mice were group-housed (n=6/group) for 2 weeks in either control (standard-housed), running (++running wheels) or enriched (+running wheels and toys) environments. Mice were then killed and their brains perfused and processed for stereological estimation of the number of tyrosine hydroxylase (TH, the rate limiting enzyme in DA synthesis) immunoreactive (TH+) SNc neurons.

Results: Mated males exhibited a significant (p<0.05, one-way ANOVA with Tukey multiple comparisons) increase, whereas mated females exhibited a significant decrease, in SNc TH+ cells (502±174 (male control), 537±48 (male mated), 566±36 (female control), 470±43 (female mated)). Environment enriched mice exhibited a significant increase in SNc TH+ cells over both running and control mice (477±78 (control), 508±73 (running), 561±58 (enriched)).

Conclusion: These data show for the first time that the number of SNc DA neurons is acutely regulated during normal behaviors in adult mice, and are consistent with the view that nigrostriatal DA can be altered in behaviorally relevant ways via activity-dependent mechanisms.

POS-MON-122

A RETRIEVAL-EXTINCTION PROCEDURE REDUCES RECOVERY OF FEAR IN ADOLESCENT RATS

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Purpose: Adolescent rats exhibit a marked recovery of extinguished fear, an impairment of extinction retention which is not evident in pre-adolescent or adult rats. A single nonreinforced exposure to the conditioned stimulus (CS; a retrieval trial) given shortly before extinction has been shown in some circumstances to reduce the recovery of fear after extinction (e.g., to reduce renewal), and is generally interpreted as a disruption of reconsolidation. The present experiments investigated whether a retrieval-extinction procedure would reduce the recovery of extinguished fear in adolescent rats.

Methods: Adolescent rats (postnatal day 34-37 at extinction, n=8-13 per group) were trained to fear a white noise paired with shock (in context A). The following day, rats received one nonreinforced CS presentation (a retrieval trial) or equivalent context exposure (no retrieval) in a second context (B) before being returned to their home cage for 10 min. Fear was then extinguished in context B via 30 nonreinforced CS presentations. Animals in the No Retrieval group received 31 CS presentations during the extinction session.

Results: The No Retrieval group exhibited a recovery of fear in the extinction context (ABB) and higher levels of fear in the training context (ABA renewal). A retrieval trial shortly before extinction reduced overall levels of fear in both test contexts (i.e., it improved extinction retention and reduced renewal). A retrieval trial 10 min after extinction produced a similar reduction in overall levels of fear as retrieval before extinction, inconsistent with a disruption of reconsolidation explanation. Finally, a retrieval trial after extinction was more effective in reducing recovery and renewal when presented shortly after extinction (10 min) than when delayed (6h).

Conclusions: These findings suggest potential manipulations to reduce the recovery of extinguished fear in adolescence.

POS-MON-123

EFFECTS OF STRESS DURING LACTATION ON MATERNAL AND OFFSPRING BEHAVIOUR

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Purpose: Stressors administered during early lactation have been shown to have significant effects on both the mothers and their offspring. Postnatal stressors can alter maternal behaviour and anxiety-like behaviors in dams and anxiety-like behavior in the offspring. The effect of corticosterone (CORT) administration, calorie restriction and noise stress administered over the first 11 days of lactation on maternal behaviours and anxiety-like behaviour in dams (n = 32), and anxiety-like behaviour and fear responses in the offspring (n = 32) were tested. Tests used include, maternal observations, pup retrieval test, elevated plus maze, open field test and predator odour test.

Results: Results found that nursing was increased in CORT rats and noise stressed rats, but not in calorie restricted rats. The effects in the CORT and noise stress rats was attributed to increases in arch back nursing but decreases in blanket nursing was also seen. The decrease in blanket nursing was also seen in the calorie restricted rats. Maternal behaviours were elevated at the beginning of the observation period and decreased at the end. Anxiety-like behaviour was increased in all groups for dams. Fear behaviour was increased in all stress groups for the offspring.

Conclusions: While not all stress groups showed increases in maternal behaviour, all groups showed higher quality nursing overall. The decrease in maternal behaviours by the end of the observation period and increases anxiety-like behaviour suggests that the stressors became chronic stressors over time. The stressors also increased fear responses in adult offspring.

POS-MON-124

PERCEPTION OF SENSATION AND CORTICOSPINAL RESPONSES FOLLOWING LOW-LEVEL TRANSCRANIAL DIRECT CURRENT STIMULATION

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Purpose: This study investigated the ability of people to discriminate the sensation between three levels of transcranial direct current stimulation (tDCS): 0.5 mA, 0.8 mA and sham. A further aim was to measure the corticospinal responses using transcranial magnetic stimulation (TMS) at intervals up to 40 mins post tDCS. Methods: Using a double blind, randomized, counterbalanced, cross over design, 5 healthy participants (3F, 2M; 26.4±4.4years) completed 3 sessions of 20 mins of constant anodal-tDCS with a 1-wk washout period between sessions. An independent person administered the tDCS using 2x25 cm2 electrodes, with the anodal electrode placed over the optimal motor area projecting to the participants’ right abductor pollicis brevis muscle, identified via TMS, and the cathodal electrode placed over the contralateral supra-orbital area. During tDCS, participants recorded their perceptions of sensation using the 11-point Pain Scale prior to tDCS, and at 1 min, 10 mins and completion of tDCS. TMS at 20% above active motor threshold was delivered prior to tDCS, and at 5, 10, 15, 20, 30 and 40 mins after tDCS whilst participants held a light tonic contraction. Results: Mean MEP at 0.8 mA progressively increased peaking at 80% above pre-values at 15 mins. MEP amplitude did not change at any time points with 0.5 mA and sham. SP duration did not change at any time points between conditions. Further, there was no difference in perception of sensation across pain and sensation subscales at any time points between conditions.

Conclusions: This study has demonstrated, in healthy people, that tDCS increases corticospinal excitability at 0.8 mA but the level of simulation cannot be identified.
**POST-MON-125**

**ODOR SPECIFIC LONG-TERM MEMORY IN HONEYBEES IS IMPAIRED BY DNA METHYLTRANSFERASE INHIBITION**

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**Purpose:** Epigenetic mechanisms are known to be involved in long-term memory formation in vertebrates and invertebrates. However not much is known about which aspects of memory are regulated by DNA methylation and what the targets are. **Methods:** Using olfactory appetitive conditioning we inhibited DNA methyltransferases at different time points before and after training using two different inhibitors, zebularine and RG108. The bees were tested for their short- and long-term memory retention of both the learned odour and an unknown odour. The expression of genes known to be involved in long-term memory formation was tested. Results: We could show that DNA methylation is an important regulatory factor during long-term memory formation, but not short-term memory formation in honeybees. The time point of inhibition was crucial as only a treatment after the training was effective in impairing long-term memory formation. Specifically the discriminatory ability, meaning the odour specificity, of the memory was dependent on DNA methylation. Both inhibitors were effective in impairing the discriminatory ability (n=40 in all behavioural experiments). Molecular analysis suggests that zebularine has a general downregulating effect, whereas RG108 acts specifically on the expression of certain targets (n=5). **Conclusion:** In sum we could show that DNA methylation inhibiting drugs impair the discriminatory ability of long-term memory retention in honeybees. The size of the effect is dependent on the inhibitor used and the time point of inhibition. Several targets were identified which show differential gene expression after treatment and are likely to be regulated by DNA methyltransferases during the formation of long-term memory.

**POST-MON-126**

**THE EFFECT OF AN AUSTRALIAN-TYPE DIET, WITH AND WITHOUT NUTRITIONAL SUPPLEMENTS, ON COGNITIVE DECLINE AND PATHOLOGY IN AN AD MOUSE MODEL**

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**Purpose:** Alzheimer’s disease (AD) is a progressive, neurodegenerative disease accounting for up to 75% of cases of dementia, the third highest cause of death in Australia. With no cure and limited treatments available, there is a social and economical urgency to find prevention for AD. **Methods:** Using an Australian-type mouse model was used to investigate the effects of an Australian-type diet, with and without nutritional supplementation, on cognitive decline and pathology in AD. **Results:** Amy mice were fed either an ideal rodent diet (AIN93-M), or a rodent diet designed to reflect the nutrient content of a typical Australian diet (Oz-AIN diet) with and without nutritional supplementation. Normal mice fed the Oz-AIN diet served as a control (n=8, all groups). **Conclusion:** There was no correlation between amyloid deposits and cognitive abilities. While nutritional supplementation was able to reduce cognitive decline, supplementation did not prevent development of amyloid deposits in the Amy mouse.

**POST-MON-127**

**CORTICOSTERONE TREATMENT DURING YOUNG ADULTHOOD DECREASES REELIN LEVELS ONLY IN MALE MICE: IMPLICATIONS FOR SCHIZOPHRENIA**

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**Purpose:** Stress exposure during adolescence/early adulthood has been shown to increase the risk for psychiatric disorders such as schizophrenia. Reelin plays an essential role in brain development and its levels are modulated independently on DNA methylation. Both inhibitors were effective in impairing the discriminatory ability (n=40 in all behavioural experiments). Molecular analysis suggests that zebularine has a general downregulating effect, whereas RG108 acts specifically on the expression of certain targets (n=5). **Conclusion:** In sum we could show that DNA methylation inhibiting drugs impair the discriminatory ability of long-term memory retention in honeybees. The size of the effect is dependent on the inhibitor used and the time point of inhibition. Several targets were identified which show differential gene expression after treatment and are likely to be regulated by DNA methyltransferases during the formation of long-term memory.

**POST-MON-128**

**SENSITISATION TO MK-801 IS ASSOCIATED WITH ALTERED Dopamine METABOLISM IN PREFRONTAL CORTEX IN THE RAT**

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**Purpose:** Dysfunction of dopamine signalling is a feature of schizophrenia as well as sensitisation to stimulant drugs, such as amphetamine, which involves remodelling of subcortical dopaminergic circuitry as well as changes within the prefrontal cortex (PFC). However, the effect of sensitisation to NMDA antagonists, such as MK-801, on dopamine release within the prefrontal cortex (PFC) remains unresolved. The aim of the present study was to examine MK-801 stimulated dopamine release within the PFC 2 weeks after a single high dose of MK-801. **Methods:** Adult Sprague-Dawley rats (N=21) were given an initial injection of MK-801 (Male; 0.5 or Female; 0.1 mg/kg). One week later microdialysis probes were implanted in the PFC under isofluorane anaesthesia, and 1 week later samples were collected for 3 hours after treatment with a second injection of MK-801. HPLC was used to assay dopamine and metabolites in in-dialysate samples. **Results:** The rats were allocated to two groups based on their locomotor response to MK-801; those that showed a sensitised response to MK-801 (n=14) and those that did not (n=9). There was a significant (P<0.05) difference in the release profiles of dopamine and the metabolites HVA and DOPAC following MK-801 treatment. Rats sensitised to MK-801 showed a significant increase in HVA and DOPAC release and altered kinetics of DA release after treatment with MK-801, compared with non-sensitised rats. **Conclusions:** This is the first study to sensitisation to MK-801 in adult Sprague-Dawley rats and the effects of dopamine metabolism in the prefrontal cortex of adult rats. Taken together, these data suggest sensitisation to NMDA antagonists may be a useful model to investigate neuronal plasticity with the PFC. Future studies are required to investigate the temporal nature of these changes and the impact on the subcortical dopamine system.
IDENTIFICATION OF THE NEURONS ACTIVATED BY AUDITORY FEAR CONDITIONING

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Purpose: To understand how memory is encoded and stored, the neurons involved in the storage process need to be identified. We have previously used fos-tau-lacZ (FTL) transgenic mice to identify neurons specifically activated in the amygdala following classical fear conditioning to a context. However, it was unclear precisely what cues in the context contributed to the fear memory. We have thus conditioned the FTL mice to exclusively fear an auditory tone and begun to map the neurons which show learning specific activation throughout the brain. This protocol should permit a more direct correlation between the behavioural components and neuronal activation patterns associated with fear memory. Methods: FTL mice were habituated to a shock chamber and exposed to an enriched environment daily over three weeks. A paired mice group then received a single paired presentation of the tone and shock. Control mice included context only, tone only and unpaired groups. Four hours after training, mice were tested for fear memory to context and tone, and then perfused. Brain sections were stained for FTL activity and neurons were identified and counted using light microscopy. Results: Only paired mice froze to tone, and insignificant freezing to context was observed. Distinct populations of learning specific neurons were identified in lateral amygdala, amygdalostriatal transition area, medial amygdala and ventromedial hypothalamus. There were significantly more FTL neurons in paired mice compared to other groups (P<0.0001). Conclusions: Neurons specifically activated by the association of tone to shock have been identified suggesting that these neurons are involved in formation of the auditory fear memory.

THE ROLE OF CYTOKINE TNF IN COGNITION-LIKE AND MOOD-LIKE BEHAVIOURS

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BACKGROUND: Cytokine TNF plays a role in development of cognitive tasks and emotional responses. TNF signals through two receptors, TNF-R1 and TNF-R2, with TNF-R1 presumably neurodegenerative and TNF-R2 neuropsychological. However, the exact role of these receptors in behavioral responses and in neurotrophin production is unknown. METHODS: Genetically modified 3 month old mice, (n=14), with TNF KO, TNF-R1 KO and TNF-R2 KO and wild type controls were subject to a behavioural battery incorporating Barnes Maze (BM) to measure cognition like behaviour, Elevated Zero Maze (EZM) to assess anxiety-like behaviour and Hole Board Exploration (HBE) for exploratory activity. ELISA was performed on hippocampus (HC) to assess anxiety-like behaviour and Hole Board Exploration (HBE) for were subject to a behavioural battery incorporating Barnes Maze (BM). We suggest a pathway through neurotrophin receptors in behavioral responses and in neurotrophin production is not exclusively neurodegenerative or neuroproliferative, as previously believed suggesting a combined effect of the 2 receptors is essential. RESULTS: Deletion of TNF caused impairment in cognition- as well as heightened anxiety like behaviour in the TNF-KO mice. Moreover, behavioural analysis of TNF R1 and TNF R2 KO mice showed that R1 and R2 pathways are not exclusively neurodegenerative or neuroproliferative, as previously believed believing suggesting a combined effect of the 2 receptors is essential in behavioural responses. We suggest a pathway through neurotrophin signaling could be a possible mechanism through which these receptors mediate cognition and mood-like behaviours.

VOLATILE ANAESTHETICS INCREASE PERIPHERAL AND CENTRAL INFLAMMATION: RELATIONSHIP TO POST-OPERATIVE COGNITIVE DYSFUNCTION

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Purpose: Post operative cognitive dysfunction (POCD) occurs in ~10% of all surgical patients but the pathogenesis is unknown. Studies in young and aged rats suggest anaesthesia and/or inflammation induced by surgical trauma may contribute to POCD. We have previously found differences between anaesthetic agents and age-dependent effects on memory in rats (Callaway et al., 2012, Anesthesiology). Here we investigated whether anaesthesia alone has any effect on markers of inflammation in blood and hippocampus in rats. Methods: Young adult (3 mo) male Sprague Dawley rats were exposed to equivalent concentrations (1 MAC, 4h) of the volatile anaesthetics sevoflurane, desflurane or no-anaesthesia condition. At 6, 24, 48h or 7d after exposure (n=3-6 per time point) rats were deeply anaesthetised (Isoflurane 5%) and blood samples taken via cardiac puncture prior to transcardial perfusion with PBS. Brains were removed and hippocampus dissected and processed for cytokine analysis. C-reactive protein was analysed in serum and cytokines were analysed in serum and hippocampus lysates (Bioplex Assay). Results: C-reactive protein levels were increased 2-fold in serum of rats exposed to volatile anaesthetics compared with no-anaesthesia controls and increases were still evident at 7d. Multiple pro-inflammatory cytokines including IL-1β (4 fold increases, P<0.001) and IL-6 (P<0.01) were significantly increased in serum 6h after exposure. Significant increases in IL-1β and IL-6 were also found in the hippocampus 6h after desflurane exposure compared with controls (P<0.02). Conclusion: Volatile anaesthetics alone cause increases in markers of inflammation in the periphery and centrally. IL-1β and IL-6 have previously been implicated in memory impairment.

THE EFFECT OF ENVIRONMENTAL STRESSORS DURING THE JUVENILE PERIOD ON ADULT ANXIETY AND FEAR BEHAVIOUR IN THE RAT

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Purpose: To investigate the effects of juvenile calorie restriction, noise stress and corticosterone administration on adult anxiety- and fear-like behaviour. Methods: Male Hooded Wistar rats (N=36) were exposed to either calorie restriction (CR; 25% restriction), noise stress (NS; 4 hours of 90-95dB white noise daily), corticosterone administration (CORT; 200μL/m in drinking water); or control conditions, from post-natal day 22 to 28. Behavioural tests of anxiety and fear were conducted when animals reached adulthood (post-natal day 60 onwards). Results: All juvenile stressed rats demonstrated decreased anxiety-like behaviour in the elevated plus maze, although not in the open field test. In the acoustic startle response test, NS rats showed a trend towards diminished startle amplitude, and reduced habituation across trials, while the CR group showed the opposite pattern. The NS and CR groups demonstrated increased startle amplitude in the predator odour-potentiated startle response test, in response to trimethylthiazoline (TMT). These groups also spent significantly less time investigating predator compared to neutral odours in the predator odour test, while the CON and CORT animals did not. Conclusion: Juvenile CR, NS, and CORT administration can have lasting effects on adult anxiety- and fear-like behaviour. These findings have implications for the understanding of the role of early life stress in the development of adult psychopathology.
POS-MON-133

MEMORY RETRIEVAL AND THE EXTINCTION OF PAVLOVIAN CONDITIONING

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Purpose: Extinction of fear learning normally yields a response decrement that is sensitive to relapse. Recent work suggests that preceding extinction training with a brief memory retrieval trial can yield a form of extinction that is insensitive to relapse. Here we studied this retrieval plus extinction manipulation in rats. Methods: Adult male Wistar rats received Pavlovian fear conditioning, extinction, and retraining. In Experiment 1 (N = 56) this involved discrete cue fear learning whereas in Experiment 2 (N=72) this involved contextual fear learning. Across both experiments we manipulated the strength of initial fear learning or relearning by varying the intensity of the footshock unconditioned stimulus. Results: In both experiments there was evidence for the acquisition of conditioned fear that was reduced by extinction training. Preceding extinction training with a memory retrieval trial had no effect on the loss of responding during extinction. Across retraining, there was evidence for rapid reacquisition of fear in the groups that had received retrieval plus extinction training. This augmentation of reacquisition depended on the strength of the initial training memory for discrete cue fear learning and the strength of the re-training memory for contextual fear learning. Conclusions: In contrast to recent findings, there was no evidence here that a retrieval plus extinction training manipulation protected animals from relapse to fear and there was evidence that under some circumstances this manipulation may increase vulnerability to relapse.

POS-MON-134

CRF1 RECEPTORS IN THE VENTRAL TEGMENTAL AREA REGULATE COCAINE-SEEKING IN MICE

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Purpose: Stress is associated with drug addiction, contributing to drug use and propensity to relapse. Corticotropin-releasing factor (CRF) has been implicated in this relationship, and the ventral tegmental area (VTA) is a suggested locus for this action. However, whether CRF within the VTA has a role in drug-seeking in the absence of acute stressors, and the receptor subtype(s) mediating this effect remains unanswered. Therefore, we assessed the effects of CRF receptor type 1 (CRF1) signaling within the VTA on cocaine and sucrose-seeking behaviour in mice using RNA interference. Methods: Mice were stereotaxically injected with lentivirus encoding short hairpin RNAs (shCRFR1 or scrambled control) targeting the VTA. The effects of this treatment were examined using an operant self-administration paradigm for cocaine (shCRFR1 virus n=27, control virus n=10) and sucrose (shCRFR1 virus n=20, control virus n=9). We then examined cue-induced reward seeking after a period of enforced abstinence. Results: Intra-VTA infusions of lentivirus-shCRFR1 had no effect on the acquisition and maintenance of self-administration of either cocaine or sucrose. However, cue-induced cocaine-seeking following abstinence was markedly attenuated in shCRFR1 treated mice compared to those receiving the control virus (p<0.01). In contrast, sucrose-seeking as not affected by intra-VTA lentivirus-shCRFR1. Conclusion: Our data suggest that CRFR1 in the VTA are critical for cue-induced cocaine seeking, but not for sucrose-seeking, suggesting the role of CRFR1 may be specific for drugs of abuse rather than rewards in general. Furthermore, it appears that CRFR1 in the VTA have a role in relapse behaviours beyond conditions that focus on stress.

POS-MON-135

IN VITRO AND IN VIVO EVIDENCE FOR THE THERAPEUTIC POTENTIAL OF CANNABIDIOIL IN ALZHEIMER’S DISEASE

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Purpose: The phytocannabinoid cannabidiol (CBD) has anti-inflammatory, anti-oxidant, and neuroprotective characterisitcs, which promise therapeutic value for Alzheimer’s disease (AD). In vitro, CBD has been shown to protect against amyloid β (Aß)-induced neuroinflammation, lipid peroxidation and neurotoxicity. Only one study to date has investigated CBD in vivo reporting beneficial effects of CBD on Aß-induced neuroinflammation. Methods: Our team investigated the effects of CBD in vitro and in vivo. CHO cells expressing human APP were treated with CBD (1μm concentration) for 24 h. Secreted Aß and cellular ßCTF were measured by western blotting and quantified after correction for total cellular Aß. Further, a transgenic mouse model for AD (APPxPS1 transgenic mice, N = 6) was treated for 3 weeks with CBD (daily i.p. injections; 20 mg/kg) post onset of AD symptoms before being tested in a cognitive test battery. Results: The in vitro data indicate that amyloid precursor protein (APP) proteolytic processing is directly modulated by CBD. CBD significantly inhibited cellular Aß production. This was associated with trends for decreased (respectively) levels of the cellular ßCTF fragment, which may indicate regulation of ß-secretase activity. Furthermore, 3-week CBD treatment of APPxPS1 transgenic mice reversed cognitive deficits in the social recognition test. Conclusions: While the mechanisms involved are unknown, these experiments highlight the necessity to investigate the potential therapeutic actions of CBD very carefully in vivo. CBD may present a novel therapeutic strategy against neurotoxicity and cognitive decline in AD.

POS-MON-136

ADENOSINE 2A RECEPTOR DELETION ABOLISHES METHAMPHETAMINE-INDUCED PLACE PREFERENCE

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Purpose: Methamphetamine (METH) is a highly addictive psychostimulant for which there are currently no therapeutic treatments. We hypothesised that METH-taking and METH-seeking behaviour may be modulated by a reduction in adenosine 2A (A2A) signalling, as 1) acute and chronic drug use alters extracellular adenosine concentrations, and 2) A2a knockout (KO) mice show reduced morphine self-administration and place preference. Methods: A2a KO mice and WT littermates were tested in conditioned place preference (CPP), locomotor sensitization and intravenous self-administration paradigms (N = 9-17 each genotype). Results: In CPP, A2a KO mice demonstrated an aversion to the METH-paired side at two doses of METH (1 and 2mg/kg), while WT mice demonstrated a preference for the METH-paired side at 2mg/kg and a neutral preference at 1mg/kg. Conditioned hyperactivity was reduced in A2a KO mice for both conditioning doses. Locomotor sensitization developed in both genotypes in a similar manner. Intravenous METH self-administration was tested using a fixed ratio 1 and 3 schedule of reinforcement and at 2 doses of METH (3 and 10µg/kg/infusion). There were no differences in self-administration behaviour under any condition. Conclusions: Reduced A2a -signalling appears to abolish the preference for a METH-paired environment, and was accompanied by a reduction in conditioned hyperactivity. This suggests a reduction in the conditioned reinforcing nature of METH in A2a KO mice, since these mice do get a CPP to cocaine. This effect appears to be paradigm specific, as A2a KO mice did not show reduced self-administration of METH in IVSA.
POS-MON-137

NOVEL INHIBITORS OF HUMAN KNYURENINE AMINOTRANSFERASE-I FOR MODULATION OF THE TRANSPORTER PATHWAY

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As an endogenous antagonist at the glutamate-binding site of the NMDA receptor, high levels of kynurenic acid (KA) has been thought to accompany the occurrence of several psychoses, including in schizophrenia. Overactivity in the enzyme human kynurenine aminotransferase-1 (hKAT-I), which catalyzes the metabolism of this metabolite, is therefore believed to be an important link in the pathophysiology of schizophrenia and makes the kynurenine pathway a valuable target for the treatment of such neuropsychiatric disease. We believe that novel inhibitors will be useful, and have been using iterations of structure-based methods to assist in the design of inhibitors of hKAT-I. In silico screening and docking calculations have been used to suggest potential inhibitors for hKAT-I, but we have also undertaken the synthesis of potential inhibitors and related synthetically accessible variants. The inhibitory effects of indole based compounds have been reported as well as a crystal structure for hKAT-I with indole-3-acetic acid bound. We report the pursuit of a series candidate of phenyl hydrazone compounds, seven of which were found to inhibit hKAT-I with the best possessing IC50 of 19.8 μM, lower than the reported indole-3-propionic acid (IC50 of 146.0 μM). To assist in further design, we have pursued crystal structures of the enzyme in complex with our inhibitors and have been able to grow crystals diffracting to 1.2 Å resolution. Currently we have a crystal structure with another well-known non selective inhibitor, aminooxy-acetic acid (IC50 of 13.1 μM) bound at 1.5 Å resolution.

POS-MON-138

ANALYSIS OF ABETA AGGREGATION STATE ON CELLS BY A NOVEL TECHNIQUE TERMED PHOTOBLEACHING IMAGE AUTO AND CROSS-CORRELATION SPECTROSCOPY

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Background: Alzheimer’s disease (AD) is the most common cause of dementia and is characterized by progressive memory loss, confusion, and cognitive deficits. Pathologically, this disease is characterized by the presence of extracellular senile amyloid plaques which are principally composed of holo and aggregates of the 39-43 residue amyloid beta (Aβ) to neurons is known. We propose to identify the oligomerization profile of Aβ42 in treated neuronal cultures using a novel biophysical imaging technique termed photobleaching with image correlation spectroscopy (pbiCS). Methods: Mouse primary cortical neuronal cells were treated with freshly prepared 6 μM FITC-Aβ42 peptide for 1, 4, and 24 hr (n=6-9) then fixed and imaged using a confocal microscope. Images were colleted in the photon-counting mode to ensure a linear intensity response. The laser beam was set to focus through a narrow pin hole. A time series mode was set up to take 15 sequential images and settings set to average 4 scans per image. Photobleaching was performed on neuronal cells for the ICS analysis on a number of different sites. The acquired images were further processed using ImageJ sofware and the analysis is tested using computer simulations on model aggregate systems and then applied to an experimental determination of amyloid beta peptide aggregation on nerve cells. Results: It is clear from these pbiCS plots that the 3 time points of the apparent CDR remaining changes with exposure time of Aβ42 to neurons in culture suggesting a time-dependent increase in Aβ peptide aggregation. Different oligomerization models were used to determine the characteristic strength of the fitting model. Globally, the datasets could be described by a monomer-dimer-tetramer-hexamer or a monomer-dimer-trimer-pentamer model. Moreover, we observed increased levels of higher order oligomers with increasing treatment times. Conclusion: The results demonstrate the utility of photobleaching with ICS for determining aggregation states on the supramolecular scale for Aβ in intact cells without the requirement for a brightness standard. The results of the study of Aβ peptide on cultured neuronal cells reveal that the aggregation states are heterogeneous and complex. Aggregation varies from cell to cell and depends on treatment time.

POS-MON-139

A PROTEOMIC ANALYSIS OF THE PREFRONTAL CORTEX IN AN ANIMAL MODEL OF METHAMPHETAMINE-INDUCED BEHAVIOURAL SENSITIZATION

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Purpose: Repeat administration of drugs of abuse, such as methamphetamine, produce a progressive increase in locomotor response to drug administration (sensitization) that is believed to mimic both the behavioural and neurochemical changes seen in mental health disorders, such as addiction and psychoses. Previous research has suggested that alterations to the prefrontal cortex (PFC) may mediate the aetiology and maintenance of these behavioural changes. The aim of the present experiment was to investigate changes to protein expression in the PFC following behavioural sensitization to chronic methamphetamine exposure. Methods: Male Sprague Dawley rats (n = 16) underwent repeated methamphetamine (1mg/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 PFC dissected out for label-free shotgun proteomic analysis using mass spectrometry (n=6). Results: Behaviourally, a methamphetamine challenge resulted in significant sensitized locomotor response in methamphetamine pre-treated animals when compared to saline controls (F<0.05). Proteomic triplicate analysis of prefrontal cortices revealed 115 proteins that were differentially altered when compared to saline controls (P<0.05). Proteomic triplicate analysis of prefrontal cortices revealed 115 proteins that were differentially altered when compared to saline controls (P<0.05). Proteomic triplicate analysis of prefrontal cortices revealed 115 proteins that were differentially altered when compared to saline controls (P<0.05). Proteomic triplicate analysis of prefrontal cortices revealed 115 proteins that were differentially altered when compared to saline controls (P<0.05). Changes in the datasets could be described by a monomer-dimer-tetramer-hexamer or a monomer-dimer-trimer-pentamer model. Moreover, we observed increased levels of higher order oligomers with increasing treatment times. Conclusion: These changes may underpin the mechanism of methamphetamine-induced sensitization and may thus serve to inform how changes to the PFC could underpin the cognitive and behavioural dysfunction commonly seen in mental illness and drug addiction.

POS-MON-140

CHRONIC INTERMITTENT TOLEUENE INHALATION DURING ADOLESCENCE IN RATS ALTERS REWARD VALUE MEASURED UNDER OPERANT CONDITIONS

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Purpose: Adolescent abuse of inhalants containing the organic solvent toluene poses a significant risk to the maturing brain and has been associated with neurobiological and cognitive abnormalities. In this study, we employed a model of adolescent toluene exposure in rats to assess the impact upon cognitive and reinforcing processes involved in operant self-administration. Methods: Adolescent (postnatal day 28) male Wistar rats were exposed to either air or chronic intermittent inhalation of toluene (CIT, 10,000ppm) 1hr/day, 3 days/week (n=10-14 per cohort). Following 4 weeks, we investigated operant self-administration of either sucrose (5% w/v; Cohort 1) or ethanol (10% v/v; Cohort 2). Results: CIT-exposure resulted in a significant (p<0.05) suppression in weight gain compared to air-exposed rats. Aspects of operant responding for a number differed between air and CIT-exposed rats with delays in acquisition and reversal learning. Cue-induced re-instatement revealed a delay in active lever responding (p<0.05) in CIT-exposed rats, although there was no difference in total lever presses when compared to air-exposed rats. There was no effect in the second differences between groups in operant responding for ethanol. Air-exposed rats displayed a significant increase in active lever responding upon reinstatement precipitated by cues previously paired with sucrose (p<0.01) compared to ethanol. In contrast, in CIT-exposed rats there was no differential between sucrose or ethanol-seeking. Conclusions: This study suggests that adolescent CIT exposure impairs aspects of instrumental learning. Furthermore, in control (air-exposed) rats the strength of the reinstatement response varied with the reinforcer previously self-administered, whereas following adolescent CIT exposure there was a similar value association between a natural and drug reward in this context.
EXTENDED EXPOSURE TO CAFFEINE AND SUCROSE HAS DIFFERENTIAL EFFECTS ON THE ORBITAL FRONTAL CORTEX PROTEOME OF ADULT SPRAGUE DAWLEY RATS

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Purpose: Caffeine is a psychostimulant that is commonly consumed by adults, often in combination with high levels of sugar. The neurobiological consequences of this combination are as yet unexplored. The aim of the current study was to investigate the effect of chronic exposure to oral caffeine (CAFF), sucrose (SUCR), or their combination on locomotor behaviour and the proteomic profile of the orbital frontal cortex (OFC) in adult rats. Method: Male adult Sprague Dawley rats, (n=12 per group) were treated for 25 days with either water, CAFF 0.6g/L, SUCR 10%, CAFF 0.6g/L+SUCR 10% or CAFF 0.6g/L+SUCR 5% in their drinking water. Locomotor behaviour was measured on the first and last day of treatment, then one week after treatment. Following final behavioural testing, brains were rapidly removed, snap frozen in liquid nitrogen then stored at -80°C until proteomic analysis of the OFC was conducted. Results: When tested drug free SUCR 10% and CAFF 0.6g/L animals were significantly more active than the rats exposed to other treatments. Label free quantitative shotgun proteomic analyses found, when compared to control, 158 and 251 proteins were differentially expressed in CAFF and SUCR rats respectively. In CAFF the top functional networks associated with changed proteins were nervous system function, cellular compromise and cell function, and in SUCR nucleic acid metabolism, signaling, energy production and DNA repair. Conclusion: These results suggest that exposure to a diet high in caffeine and sucrose has the potential to change behaviour and neural functioning of the OFC.

THREE NOVEL POTENT AND EFFICACIOUS POSITIVE ALLOSTERIC MODULATORS OF THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR EXHIBIT COGNITIVE ENHANCEMENT IN RODENT BEHAVIOURAL MODELS

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Positive allosteric modulators (PAMs) of the α7 nAChR are an emerging category of drugs for treatment of cognition/memory impairing CNS disorders such as Alzheimer’s disease and schizophrenia, which potentially offers advantages over traditional full or partial α7 agonists. Three novel α7 nAChR PAMs, BNC1881, BL362, and BL343, were identified in Bionomics’ α7 discovery program by using high throughput influx-based screening and an automated patch clamp platform, Patchliner. The compounds were further characterized in detail by conventional patch-clamp using a fast application system Dynaflow. BNC1881, BL362, and BL343 tested at 3 μM produced 421 ± 86%, 1131 ± 20% and 533 ± 205% (n = 3-4) potentiation of acetylcholine-induced currents respectively. Full dose-response curves were obtained for BNC362 and BL343 with EC50 values of 1.9 (n = 1.62) and 1.8 μM (n = 1.66) respectively. Cognition-enhancing properties of novel PAMs were evaluated as reversal of scopolamine-induced memory deficits in T-maze Continuous Alternation Task (T-CAT) (BL362 and BL343, n ≥ 10, mice) and in novel object recognition model (NOR) (BNC1881, n = 12, rats). BNC1881 significantly reversed scopolamine-induced (1 mg/kg) deficits and fully restored performance to that of the vehicle-treated rats in the NOR at 3 and 10 mg/kg (i.p.). Oral dosing with BL362 and BL343 exhibited dose-dependent reversal in T-CAT (1-30 mg/kg, p.o.). In summary, we have discovered three novel α7 nAChR PAMs which potently enhance α7 nAChR-mediated currents in vitro and reverse scopolamine-induced memory impairment in two rodent models in vivo when administered intraperitoneally or orally.

ADULT VITAMIN D DEFICIENCY LEADS TO BEHAVIOURAL AND BRAIN NEUROCHEMICAL ALTERATIONS IN C57BL/6J AND BALB/C MICE

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Purpose: Epidemiological evidence suggests that vitamin D deficiency may predispose people to develop depression and cognitive impairment. However there is a lack of research examining the effects of adult vitamin D (AVD) deficiency on brain outcomes. The aim of this study was to examine the impact of AVD deficiency on behaviour and brain function in mice. Methods: Adult male C57BL/6J and BALB/c mice were fed a control or vitamin D deficient diet and assessed on a broad range of behavioural domains (n=28/diet/strain), psychomimetic-induced locomotion in response to D-amphetamine and MK-801, and neurotransmission in brain tissue (n=8/diet/strain). Results: Overall, AVD deficiency resulted in hyperlocomotion in a novel open field and reduced GAD65/67 levels associated with AVD deficiency may be relevant to a number of neuropsychiatric conditions, including schizophrenia and depression. This is the first study to show an association between AVD deficiency and prominent changes in behaviour and brain neurochemistry in the mouse.

IMPAIRMENTS IN THE ONTOGENY OF DOPAMINE SYSTEMS PRODUCE BEHAVIOURAL RESPONSES IN LARVAL ZEBRAFISH ILLUSTRATIVE FOR NEUROPSYCHIATRIC DISEASE

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Introduction: Dysregulation of the neurotransmitter dopamine (DA) in the central nervous system is hypothesised to precede and influence the course of neuropsychiatric disorders. Previous work has shown that decreases in DA production during early development have led to anxiety-related behaviour and attenuated startle response in adult fish. The current study aims to produce high throughput screening assays to test the effects of decreased developmental DA on well-established brain functional measures such as startle response, and to establish a measure of sensory motor gating (Prepulse-inhibition). Methods: The effect of diminishing pre-pulse interval and intensity will be examined at startle responsiveness in six days post fertilisation (dpf) larvae. DA synthesis will be reduced in using exisent Morpholino Oligonucleotides (MO) in zebrafish embryos, designed to target the Tyrosine Hydroxylase (TH) gene. Startle response latency, head turn angle, and peak angular velocity will be outcome measures. Results: To date we have a reliable startle pulse of 5ms at 200Hz in 6dpf larvae (86.33% startle, N=151). We confirm that MO exposure reduces both TH production (N=30, F(2,8)=20.4, p< 0.01) and DA synthesis (N=30, F(2,11)=17.5, p<0.05) in 6dpf larvae. PPI studies are ongoing. Conclusions: Our previous studies have established that early alterations in DA ontogeny affect behaviour in adult zebrafish. This work may validate the use of Prepulse-inhibition as a useful high throughput method for testing behaviours considered endophenotypes of neuropsychiatric disorders.
POS-MON-145

SEPTAL GLUCAGON-LIKE PEPTIDE 1 RECEPTOR SIGNALING IS CRUCIAL FOR BEHAVIOURAL EFFECTS OF COCAINE

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Background: The Lateral Septum (LS) is stimulated by a number of drugs of abuse, and plays a key role in the contextual reinstatement of drug-seeking. However, the molecular mechanisms underlying neuroplasticity in the LS are not well understood. The glucagon-like peptide 1 receptor (GLP-1R), a G-protein-coupled receptor, is expressed in various CNS regions including the LS. Central stimulation of GLP-1R suppresses food reward by interacting on the mesolimic system. GLP-1R agonists can cross the blood brain barrier but it is not known if systemic administration activates septal GLP-1R.

Purpose: To determine the expression of GLP-1R in the mouse brain we performed in situ hybridisation studies and confirmed most abundant expression of GLP-1R mRNA in the LS. Systemic administration of the receptor agonist (Ex-4, 2ug/kg) triggers neuronal activity in the LS determined by c-fos-immunohistochemistry. We used the locomotor sensitization to cocaine (10mg/kg, i.p.) to study enduring neuroplasticity in addiction. Pharmacological blunting of GLP-1R signalling with the specific antagonist (Ex-9-39, 150ug/kg, i.p.) in C57/B16 mice (n=7) as well as the complete loss of GLP-1R in GLP-1R KO mice (n=12) attenuates the behavioural effects of cocaine. We complemented GLP-1R specifically in the LS of adult GLP-1R KO mice using neurotropic adeno-associated virus (AAV)-mediated GLP-1R gene transfer. AAV/12-GLP-1R was delivered bilaterally into the LS of GLP-1R KO mice (n=12). As controls, fire-matched AAV-EGFP virus was injected into the LS of wildtype (n=12) or KO animals (n=12). 3 weeks post surgery, when AAV-mediated transgene expression has peaked to remain at stable levels, animals were subjected to the sensitization paradigm. AAV-GLP-1R-injected KO animals showed a wildtype-like performance.

Conclusion: Our data suggest a pivotal role of GLP-1R signalling in the LS in addiction.

POS-MON-146

THE MGLUR5 ANTAGONIST MTEP AFFECTS THE ACQUISITION OF THE EXTINCTION OF ALCOHOL-SEEKING

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Purpose: Extinction learning in several conditioning preparations depends on glutamatergic neurotransmission, but the role of glutamate in extinction of drug seeking, and the specific receptors involved, remain poorly understood. Recent evidence has identified a role for metabotropic glutamate receptors in drug seeking behaviours. We investigated the role of mGlur5 and NMDA receptors in the acquisition and expression of the extinction of alcohol-seeking as well as the neuroanatomical locus for this effect. Methods: Male Long-Evans (n=8-12 per group) were trained in 1 hr sessions across 7 days to respond for alcoholic beer. Following this, animals underwent extinction training during which responding was no longer reinforced. Results: In Experiment 1, injection of the mGlur5 antagonist MTEP (1 mg/kg) impaired the acquisition but not expression of extinction. In contrast, in Experiment 2a injection of the NMDA receptor antagonist ifenprodil (3 mg/kg) and Experiment 2b the NMDA receptor antagonist MK-801 (0.1mg/kg), did not impair extinction learning. In Experiment 3 the impairment of extinction learning by MTEP was shown to be dose-dependent (0-3mg/kg). In Experiment 4 post training injections of MTEP (2mg/kg) did not impair the acquisition or expression of extinction learning. Finally, Experiment 5 studied the role of basolateral amygdala (BLA) mglur5 receptors in extinction learning by microinjecting MTEP into the BLA prior to extinction training. Taken together, these experiments identify a role for mGlur5 receptors in extinction learning during drug self-administration.

POS-MON-147

THE EFFECT OF CHEMOKINE RECEPTOR SIGNALLING ON BEHAVIOUR

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Purpose: Inflammation is regarded as an important mechanism of neuropsychiatric disorders. Chemokines, which are a part of the immune system, have effects on various aspects of brain function, but little is known about their effects on behaviour. Methods: We have compared the cognition-like behaviour (learning and spatial memory) of CCR6-/-, CCR7-/- and CCR6-/-/CCR7-/- mice, in the Barnes maze, which involves training mice to enter an escape box in a large open platform. We have also looked at a range of other behaviours, including exploratory, anxiety and depression-like behaviour, using a battery of tests in CCR6-/-, CCR7-/- and WT mice.

Results: In the Barnes maze, CCR7-/- mice were shown to take longer to learn the extinction learning during drug self-administration.

Conclusions: These results suggest that chemokine receptors may play a role in cognition and learning behaviour, as well as anxiety and other behaviours, although the biological mechanisms are unclear at this stage.

POS-MON-148

DETERMINING THE KEY NEUROTOXIC Aβ OLIGOMERIC SPECIES ASSOCIATED WITH ALZHEIMER’S DISEASE

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Background: Alzheimer’s disease (AD) is the most common form of dementia in aging people representing 60-80% of dementia cases. AD causes progressive neuronal loss in the cortex and hippocampus of the brain which leads to symptomatic memory impairment and cognitive dysfunction. The pathological hallmarks of AD are extracellular Aβ plaques and intracellular tau neurofibrillary tangles. Aβ plaques were formerly regarded as the toxic species in AD. However, recent evidences showed that soluble oligomeric Aβ species had better correlation with the severity of AD compared to Aβ plaques in AD brains. This suggests that soluble oligomeric Aβ species are the key neurotoxic species. The exact oligomeric species which is responsible for causing the neurotoxicity associated in AD still needs to be determined. Clearly, Aβ binding to neuronal cell membrane is a key neurotoxic event that will lead to neuronal cell death. Therefore, our working hypothesis is that Aβ toxicity correlates with the binding of a specific oligomeric Aβ species on the neuronal plasma membrane. Methods: Cell binding and microarray assays of synthetic Aβ42 and Aβ40 peptides were performed at 2 different doses for different treatment times (10 min to 96 h) to determine the kinetics and correlation of Aβ binding and neurotoxicity using mouse primary cortical neuronal cultures. Each experiment was repeated at least 3 times. Results: The degree of Aβ binding to neurons was time-dependent and Aβ-induced neurotoxicity was dependent on the concentration of Aβ bound to neurons. We calculated the minimum neuronal binding concentration to induce significant neurotoxicity was 80 pM/cell (n=13) for Aβ42 and 100 pM/cell (n=4) for Aβ40. In addition, a significant correlation was identified between the binding concentration for the trimer (R2=0.266; p<0.001) and tetramer (R2=0.409, p<0.001) Aβ42 oligomeric species and cell viability. Conclusion: The identification of the trimers and tetramers as the potential neurotoxic Aβ oligomeric species will assist us in the future development of AD therapeutic agents with more specific targets in the brain of AD patients.
M1 MUSCARINIC RECEPTOR-MEDIATED CELL DEATH IN HEK293-M1 CELLS

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Purpose: The M1 muscarinic acetylcholine receptor (mACHR) has been identified as a potential therapeutic target in Alzheimer’s disease (AD) following evidence for a role in cell survival signalling in a variety of cell types. To investigate M1 mACHR-mediated cell survival we created a stable HEK293 cell line expressing the human M1 mACHR. Methods: HEK293 cells expressing human M1 mACHR were grown in 96 well tissue culture plates. Cell survival was examined using xCELLigence technology and counting of Hoechst-labelled cells. Immunocytochemistry was used to examine expression of M1 mACHR and EGR-1, and phosphorylation status of ERK and CREB. The Discovery-1 automated microscope and MetaMorph software were used for image collection and analysis. Results: The expressed M1 mACHR correctly localised to the cell surface and was rapidly internalised following exposure of the cells to the M1 mACHR agonist carbachol (100 µM). Furthermore, early signalling cascades involving phosphorylation of ERK and CREB and activation of the transcription factor EGR-1 was reported as reported in previous studies. However, significant cell death was seen within 24 hours of the addition of carbachol (p<0.0001). Blockade of ERK signalling with 5 µM U0126 did not prevent cell death (p>0.0001). Conclusions: A stable HEK293 cell line expressing human M1 mACHR was successfully created. Activation of the M1 muscarinic receptor mediates rapid cell death in HEK293 cells through an ERK-independent pathway.

ROLE OF TAM RECEPTOR SIGNALLING IN CENTRAL NERVOUS SYSTEM MYELINATION

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Purpose: The TAM receptor family of tyrosine kinases, comprising Tyro3, Axl and MerTk, has been shown to have a critical role in the outcome of demyelination. We have previously shown that the TAM ligand Gas6 directly increases myelination in vitro and hypothesise that this is mediated through Tyro3. We therefore investigated the role of Gas6 in myelination of the CNS using mice deficient in Gas6. Further, based on ChIP sequencing data identifying potential myelin gene regulatory factor (MRF) binding sites within the Tyro3 gene, we examined the role of MRF in Tyro3 gene expression. Methods: We used an in vitro luciferase assay to test the role of MRF in regulation of Tyro3 gene expression. Electron microscopy was used to analyse myelin thickness and the number of myelinated axons in the optic nerve and corpus callosum of 3 month old Gas6–/– and WT mice (n=3–4/group). Results: Using in vitro luciferase assays, we did not detect any direct impact of MRF on Tyro3 gene expression. Furthermore, no significant differences were observed in g-ratios, myelin thickness or number of myelinated axons between Gas6–/– and WT mice at the 3 month timepoint. Conclusions: Although we have previously shown that Tyro3 expression is increased upon oligodendrocyte differentiation, this does not appear to be mediated directly by MRF, a major regulator of myelin gene expression, indicating that Tyro3 gene expression may be under the control of other factors. Consistent with previous findings, we observed no differences in myelination in adult mice. Whether, however, this process is affected in early postnatal development of Gas6 deficient mice remains unknown and will be investigated in both Gas6–/– and Tyro3–/– mice.

CYCLOSPORINE AFFECTS GLIAL SCAR FORMATION IN ENDOTHELIN-1 STROKE MODEL

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The effect of cyclosporine A (CsA) on stroke outcome including subsequent inflammatory response and mechanisms of repair such as angiogenesis has yet to be determined. Purpose: To investigate the effects of CsA administration prior to stroke on neurological and histological outcomes 7 days post-stroke using the endothelin-1 (ET-1) rat stroke model. Methods: Administration of ET-1 induced stroke by causing constriction of the right middle cerebral artery in conscious rats. CsA (10mg/kg, n=8) or vehicle control (2.2% ethanol; 0.9% castor oil in saline, n=8) was administered 2 days prior to stroke and everyday thereafter. Neurological and histological outcome was assessed by neurological deficit score and MCID image analysis. Blood vessels, activated microglia/macrophages, and astrocytes of activated and severely diffuse morphologies were immunohistochemically detected using von willebrand factor (vWF), OX42 (CD11b) and glial fibrillary acidic protein (GFAP) and point counted using the CAST system. Angiogenesis and inflammation was assessed in the infarcted cortex, striatum and surrounding border zones and compared with the corresponding mirror images on the contralateral side. Results: CsA treatment significantly reduced neurological deficits compared to vehicle treated at 48 hrs post-stroke but not later (P<0.05). Histopathology revealed no significant difference in infarct volume in the cortex and striatum between treatment groups. CsA administration significantly reduced the number of astrocytes with severely diffuse morphologies in the surrounding cortical (P<0.05) and striatal (P<0.05) border zones. CsA treatment appeared to have no affect on angiogenesis or activation of microglia/macrophages 7 days post-stroke. Conclusion: CsA administration prior to stroke appeared to reduce glial scar formation and astrocytes displayed activated morphologies which have been suggested to have beneficial effects on neurons.

OLIGODENDROCYTE DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS DERIVED NEURAL PRECURSORS

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Purpose: In the mammalian central nervous system (CNS), myelination of axons by oligodendrocytes is essential for fast electrical propagation. Following CNS injury, loss of mature oligodendrocytes, produce profound neurological disability. Human embryonic stem cells (hESCs) may provide a promising option to generate transplantable oligodendrocytes to remyelinate preserved axons after injury. The purpose of this study was to derive a high purity population of homogeneous oligodendrocytes from hESCs in an attempt to limit the generation of oligodendrocytes, which may contribute to a glial scar post-transplantation. Methods: The hESC lines, hES3, Me11, H9 and the human reporter cell line NKX2.1 have been used for differentiation. Differentiation to oligodendrocytes was induced using a standardised growth factor-dependent protocol with modification. The expression of neural and oligodendrocyte markers have been examined following 5, 8 and 12 weeks of differentiation respectively, using flow cytometry and immunocytochemistry. Results: The different hESC lines, H9, Me11 along with the NKX2.1 reporter cell line, have shown variable oligodendrocyte differentiation results (n=1 per hESC line, with ~n=150 spheres per well). The highest yield of oligodendrocyte precursor cells (OPCs), were generated using the NKX2.1 reporter cell line (60% CD140a- and NG2-positive OPCs). We were unable to obtain more than 1% of O4-positive pre-myelinating or MBP-positive mature oligodendrocytes. Immunocytochemistry analysis also revealed the expression of the common OPC transcription factors NKX2.2, Olig2 and SOX10. Conclusion: The data suggest that different hESC lines show disparate oligodendrocyte differentiation potential using standardised protocols. Furthermore, the NKX2.1 reporter cell line may be an excellent choice in deriving a more homogeneous population of OPCs.
POS-MON-153
IMPACT OF ENVIRONMENTAL ENRICHMENT (EE) ON SENSORY CORTEX
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Purpose: An enriched social and physical environment has been shown to produce many molecular, anatomical, electrophysiological and behaviour benefits even in adult animals. The electrophysiology linking the molecular and anatomical changes in cortex to behaviour has only been moderately well studied and has generally been restricted to neurons only in particular laminae (input laminae in the case of sensory cortex). This constrains the ability to understand how EE-induced changes affect the network processing of input through intra-cortical processing to output in a cortical area. We investigated EE-induced changes in neuronal encoding of sensory stimuli across all laminae of the rat barrel cortex that receives input from the face whisker tactile system. Methods: Animals were assigned to either ‘isolated’ (n=13) or ‘Enriched’ (n=15) housing for 8-10 weeks. In isolated conditions, animals were housed individually in standard 31 x 44 x 25 cm cages. Animals in Enriched conditions were housed in groups of 3 in 69 x 60 x 270 cm runs which were enriched with a variety of ‘toys’ kept constant for the same period. Throughout the experimental period, extracellular recordings were obtained from barrel cortex in response to whisker motion mimicking a variety of those seen in awake animals undertaking different tasks. Results: There were significant differences in cortical excitability between the groups. Enrichment resulted in increases in neuronal responses to all stimuli, ranging from those modelling exploratory behaviour through to discrimination behaviours. These increases were seen throughout the cortex from supragranular layers through to input Layer IV and for some stimuli, in infragranular Layer V. Conclusion: Long-term enrichment induces significant changes in population responses mainly in integrative and input sensory cortical layers, indicative of increased cortical plasticity.

POS-MON-155
NATURAL DELETION OF LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTOR A3 MAY BE ASSOCIATED WITH CLINICAL OUTCOMES OF MULTIPLE SCLEROSIS
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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Despite extensive study regarding immunopathogenesis and genetic determinants, factors regulating clinical outcomes are poorly understood. Leukocyte immunoglobulin-like receptor (ULIR) A3 is a soluble member of a new family of surface receptors on leukocytes that have key immune regulatory functions. Recently, for the first time we found abundant expression of A3 in normal primary CNS neurons, where it may promote neurite outgrowth. Interestingly, recent studies showed that natural deletion of A3 gene is associated with increased incidence of MS. Collectively, these findings suggest that A3 may play a critical role in the pathogenesis of MS. Purpose: Given the clinical effects of MS are primarily due to immune-mediated demyelination and axonal degeneration, we postulated that deletion of A3 may contribute to inability of the damaged neurons to regenerate leading to poorer clinical outcome. Methods: We purified genomic DNA from patient sera obtained from Accelerated Cure Project for Multiple Sclerosis, USA. PCR-based method was used to determine A3 gene deletion in 219 patients with relapsing remitting (RRMS), 112 with secondary progressive (SPMS) and 33 with primary progressive (PPMS). A3 protein in each subject was determined by ELISA. A3 genetic profile and protein levels were then correlated to disease pattern. Results: Homozygous A3 deletion was detected in 6.1% of PPMS, 1.8% of SPMS and 3.2% of RRMS. As expected, patients with wild type A3 had higher level of protein as compared to patients with heterozygous deletion in serum. Conclusion: We showed that homozygous A3 deletion was significantly more prevalent in patients with PPMS as compared to the RRMS, suggesting that lack A3 may contribute to severe disease pattern due to poor neuronal regeneration after immune-mediated injury. Restoring normal levels of A3 in patients may contribute to improved clinical outcome.

POS-MON-154
A SYSTEMATIC GENOME-WIDE SCREEN FOR DE NOVO VARIANTS IN THE SYNAPSE-GENE NETWORK OF AUTISM SPECTRUM DISORDERS USING GENOMIC SEQUENCING TECHNOLOGY AND NETWORK ANALYSIS
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Autism spectrum disorder (ASD) is the neuropsychiatric disorder with cognitive deficits and impaired brain plasticity. However, genetic heterogeneity is a major challenge to understand the aetiology of ASD and determine how (or how much) candidate genes play a role in a molecular pathway in the nervous system. Here we demonstrated disease-network analysis combined with genomic sequencing technology reveals causal variants disrupting synapse transmission and neurosignalling complex. In the method, we collected the whole blood from 32 individuals, which are 8 families with each family consisting of offspring with ASD and their parents, except one family absent in father. We performed the second generation sequencing on the whole protein-coding regions and additional intergenic, UTRs, and pre-microRNAs sequences (64Mb). Using computational algorithms, we examined variants (SNPs and indels) (SNPs and indels) <100bp called from sequencing data and then filtered them by previously characterized as common or non-causal variants in other databases and inheritance from parents. To integrate de novo variants and the synaptic gene network, we use public databases and literatures to retrieve primary ASD candidate genes followed by appending them to protein-protein interaction network. In the result, we found 8 de novo SNPs& indels and 6 CNVs. In the network analysis, 14 primary variants were revealed to connect with other 22 ASD candidate genes previously known and 37 neural pathways. Our data support the notion that perturbations in the synaptic genes contribute to the pathogenesis of ASDs, and it furthermore provides genetic-background in clinical implication based on brain imaging, diagnostic marker or pharmacological targets.

POS-MON-156
STEM CELL-DERIVED HUMAN NEURONS TO SCREEN FOR NEUROPROTETANTS: BENEFIT FROM HYPOTHERMIA BUT NOT NYX-059
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Background: Despite many neuroproaptants working in animal models of stroke, none work in the clinic. While stem cell based therapies could be of benefit, an alternative use of stem cells is to create an in vitro screening system in which human embryonic stem cells (hESCs) differentiated into neurons are used to test candidate drugs. This study aimed to differentiate hESCs into neurons, develop models of ischaemic injury and test candidate therapeutics. Methods: hESCs were differentiated into neurons in the presence of bone morphogenetic inhibitor protein and Noggin. The neurons were maintained for 11 days to mature prior to the induction of injury. Three injury models were used: Oxygen deprivation (OD), oxygen- glucose deprivation (OGD) and oxidative stress (H2O2). Two candidate therapeutics (hypothermia and NYX-059) were tested and cell death was quantified using a lactate dehydrogenase assay (LDH). Results: Hypothermia to 33°C reduced H2O2 and OGD induced cell death by 53% and 45% respectively at 24h. The neuroprotective effect of hypothermia diminished with time however it was neuroprotective even when administered 6h after H2O2 induced cell death and only short duration exposure was required. Hypothermia provided no protection against oxygen deprivation alone. NYX-059 provided no effect on neuronal cell survival in any of the injury models. Conclusion: These results demonstrate that hESCs have the potential to be a useful model for drug screening. Identifying neuroprotective agents that work in such human in vitro systems may bridge the gap between animal studies and clinical trials thereby addressing the current translational failure.
POSTERS

ALPHA SYNUCLEIN IS A MAJOR REGULATOR OF IRON HOMEOSTASIS

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Purpose: Nigral iron accumulation may play an important role in Parkinson's disease pathogenesis. We recently reported in Cell and Nature Medicine the participation of major neurodegenerative disease proteins, APP and tau, in iron homeostasis. Since the Parkinson's protein, alpha synuclein (asyn) is a ferric reductase and its translation is regulated by iron, we hypothesized that asyn, too, is involved in iron homeostasis. Methods: We decided to investigate the iron status in asyn KO mice. If the asyn knockout phenotype is, like tau KO and APP KO, dependent on advanced age, pathology may not be evident in young mice. We investigated in 15 month old asyn KO and background mice (n=14 each): behavior (Rotarod, Open field, y-maze) iron status (ICP-MS, Laser ablation ICP-MS, hematology) and neuroanatomical features (SN neuron number, Lateral ventricular area, MRI). Results: asyn KO mice exhibited highly significant t-tests: 0.0005×P<0.05→) iron depletion compared to age-matched controls in substantia nigra (-28%), hippocampus (-32%), cerebellum (-23%), cortex (-11%) and liver (-40%). Asyn KO were also anemic with low hemoglobin, MCV, MCH and MCHC (-30%). Asyn KO mice exhibited motor disability (Rotarod, open field), cognitive disability (Y-maze). Disruption in asyn KO mice was associated with neurodegeneration: SN neuron loss (-20%) and increased lateral ventricular size (+69%). Conclusion: These observations have the potential to re-orient our understanding of asyn in health and disease. Our data demonstrate that loss of asyn disturbs iron homeostasis, engenders neurodegeneration. These data would argue that the current therapeutic aim of lowering asyn in PD may be counterproductive and may have off-target affects in the periphery and the brain.

HUMAN AMNION EPITHELIAL CELLS ATTENUATE VENTILATION-INDUCED BRAIN INJURY IN PRETERM LAMBS

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Purpose: Preterm infants often require mechanical ventilation in the delivery room. However, many are inadvertently exposed to high tidal volumes (V), which can cause white matter (WM) inflammation and injury. We determined whether human amnion epithelial cells (hAECs) can attenuate brain injury resulting from high V ventilation in preterm lambs. Methods: Lambs at 0.8 gestation were delivered and ventilated for 2h with an initial high V (15mL/kg) for 15 min followed by a protective V (7mL/kg) for 105 min. At 5 min, lambs were randomised to receive intravenous and endotracheal administration of labelled hAECs (90×10^5 cells/3ml; n=5) or phosphate buffered saline (control; n=5). Unventilated controls (UC, n=5) were used for comparison. Brains were immunostained to identify microglia (anti-Iba-1) and blood-brain-barrier permeability (anti-sheep serum). The areal density of microglia and the extent of serum extravasation were assessed in the cerebral white matter (WM) in all groups. Results: There was no difference (p>0.05) in body weight, brain weight or brain-to-body weight ratio between groups. Cerebral oxygenation was not different during ventilation between groups (p>0.4). Inflammatory ventilation increased the density of Iba-1-immunoreactive microglia in the periventricular WM of the frontal lobe; hAEC administration prevented the increase (p<0.05). Permeability of the blood-brain-barrier appeared to increase following injurious ventilation and hAEC administration prevented this increase. Conclusion: High V ventilation causes brain inflammation and a loss of blood-brain-barrier integrity in the preterm ovine brain and these alterations are attenuated using hAECs. These results highlight potential therapeutic use of hAECs in preterm infants that require mechanical ventilation.

ARE CHRONIC DISORDERS PRODUCED BY ENVIRONMENTAL PHYSIOLOGICAL STRESS (ETPS) DIRECTLY INDUCED BY CELL SIGNAL PROTEINS WITH CYSTEINE CLUSTER MOTIFS CONSTITUTING SPECIFIC TOXIC (NOXIPATHIC) PATHWAY

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For the last fifty years a set of neuroautonomic-muscular-neuroinflammatory-neuropsychiatric disorders (NAPMI) including headache, fatigue, muscle pain disorders, cognitive loss, anxiety eg radiotherapy, chemotherapy and proinflammatory pharmaceutical treatment. A small group cell signal proteins and genes including MAPK p38 c-Jun c-Src p53 which are stress responders as they are affected by heat, oxidative stress, osmotic change, viral infections is cellular equivalent of ETPS, were recently found to also have the specifically conserved cysteine cluster motif adjacent to kinase (SCCCMAK) which are affected by s-nitrosylation (sNO) often found in inflammation. So therefore one logically hypothesises that SCCCMAK proteins could constitute specific dedicated toxic NOxicopathic pathway triggered by ETPS. PURPOSE OF REVIEW: To conduct preliminary survey of above hypothesis that the neurodysfunctional disorders (NAPMI) which are associated with environmental stress are directly induced by small number of signal proteins with SCCCMAK. METHODS To use a literature meta-analysis of gene assays etc. to ascertain if there is any association between forty major NAPMI disorders with environmental nitrosative triggers, signal transducers with SCCCMAK and loss of innate protection. Analysis of control group of non-inflammatory disorders for evidence of oxidative or nitrosative stress. RESULTS: Broad association of forty neurodysfunctional disorders is associated with environmental triggers as well as specific cell signal changes due to SNO or ETPS. Controls - no strong relationship found. CONCLUSION There is evidence for the preliminary hypothesis that in the absence of innate antioxidant neuroprotection, then signal proteins with SCCCMAK can induce NAPMI diseases thereby constituting a specific NOXicopathic pathway induced by pathophysiological trauma.

EFFECTS OF TREADMILL TRAINING ON HINDLIMB MUSCLE PROPERTIES IN SPINAL CORD INJURED MICE

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Muscle fibre type conversion and atrophy is observed in animal models of spinal cord injury (SCI). Exercise is known to prevent some of these changes, however, the training duration required to optimize recovery has not been investigated. Purpose: To examine the effect of 3 and 6 wks of exercise on hindlimb muscle properties after SCI. Method: Adult mice (C57Bl/6) received a left spinal hemisection and then were assigned to untrained and trained (10 min treadmill, 5 x wk for 3 or 6 weeks) groups. Mice were sacrificed (100 mg/kg; i.p. ketamine, decapitation) and the medial gastrocnemius (MG), soleus (SOL) and tibialis anterior (TA) muscles were removed from left (injured) and right (uninjured) hindlimbs. ATPase histochemistry was used to assess muscle properties. Digital images were captured and analysed with Image J software and data were compared using analysis of variance and Tukey post hoc tests. Significance was set at P > 0.05. Results: Fiber type composition and fiber area were not altered in SOL and TA muscles in either limb of 3 (n = 4) or 6 wks (n = 4) trained and untrained animals. Fibre type composition was also unaltered in MG, however, fibre type IIB area was 33% larger in MG from the injured-limb vs. untrained injured-limb after 6 wks of training. The area of type IIX fibres was also larger in MG muscles from the injured vs. uninjured side in 6-wk trained animals. Conclusion: Six weeks of treadmill training selectively reduces atrophy in type IIB and IIX fibres in the fast twitch gastrocnemius muscle.
**POSTERS  Monday**

**POS-MON-161**

**Ghrelin mediates the neuroprotective effects of calorie restriction by activating a novel AMPK-Parkin model of Parkinson’s disease**

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Purpose: Ghrelin restricts degeneration in Parkinson’s disease (PD) and calorie restriction (CR) increases plasma ghrelin concentrations. We hypothesized that elevated ghrelin during CR prevents neurodegeneration in PD. Methods: CR or ad libitum (ad-lib) ghrelin wild-type (WT) and ghrelin knockout (KO) mice were given MPTP to model PD. We counted tyrosine hydroxylase (TH), astrocyte (GFAP) and microglial (Iba1) cell number in the substantia nigra (SNpc) and striatal dopamine or metabolites by HPLC. To understand how ghrelin restricts degeneration in CR, we measured AMPK phosphorylation (pAMPK) in the SNpc and striatum. We also examined the interaction between pAMPK and Parkin or PINK1 in the SNpc and striatum using immunoprecipitation.

Results: CR attenuated TH cell loss relative to ad-lib in WT and not KO mice treated with MPTP, suggesting increased plasma ghrelin mediates the neuroprotective effect of CR. CR prevented the increase in GFAP astrocyte cell number observed in ad-lib WT mice after MPTP treatment. KO mice exhibited a significant increase in GFAP astrocytes after MPTP treatment, which reflects a need to remove more degenerating TH neurons. There were no differences in microglial activation. AMPK is a downstream target for ghrelin in the hypothalamus and our results show that CR activates pAMPK in the SNpc and striatum in a ghrelin-dependent manner. We identified that pAMPK binds to Parkin in the SNpc and striatum, although total Parkin protein levels do not change.

Conclusions: Our data reveals that ghrelin mediates the neuroprotective effects of CR on the nigrostriatal dopamine system. We have identified an interaction between pAMPK and Parkin and hypothesise that ghrelin activates this pathway to optimise mitochondrial function and reduce degeneration.

**POS-MON-162**

**Knock-down of MIP-1α in mice with the lysosomal storage disorder MPS IIIA does not lessen disease severity**

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Mucopolysaccharidosis IIIA (MPSIIIA) is a neurodegenerative lysosomal storage disorder (LSD) resulting from a mutation in the sulphamidase gene. Sulphamidase activity is reduced and its substrate, heparan sulphate (HS) accumulates. Children with MPSIIIA exhibit profound cognitive impairment and in the absence of a treatment, commonly die in their teens. To elucidate the basis of symptoms and develop therapies, we have examined neuropathology in MPSIIIA mice. HS accumulation in brain is accompanied by early/progressive neuroinflammation.

**Purpose:** to determine whether disease-course in MPSIIIA mice could be modified by modulating neuroinflammation, we knocked out macrophage inflammatory protein-1α (MIP-1α), a macrophage recruitment signal. This strategy delayed symptom onset/neuroinflammation in mice with the LSD Sandhoff disease1. **Methods:** the effect on open-field exploration and cognition in the Morris water maze (MWM) (n=15/group), and in a subset of these mice neuropathology, was compared with that in MPSIIIA/MIP-1α+/-, MPSIIIA/MIP-1α-/-, or unaffected mice. **Results:** the performance of MPSIIIA/MIP-1α-/- mice in the open-field and MWM declined when compared with that of MIP-1α+/- and MPSIIIA/MIP-1α+/- mice. As expected, engagement of the endo/lysosomal system, determined via quantification of lysosomal integral membrane marker-II staining was observed in all MPSIIIA groups (p<0.05), with no differences between these groups. There was no difference in the number of isolectin B4-stained activated microglia in the MPSIIIA groups (p>0.05).

**Conclusion:** our findings support those in another LSD, Niemann-Pick C2, where exacerbation of disease was observed when MIP-1α was deleted. Increased MIP-1α in the MPSIIIA brain may not influence recruitment of peripheral macrophages. 1. Wu and Proia (2004) Proc.Natl.Acad.Sci. USA 101:8425-8430. 2. Lopez et al. (2012) Hum Mol Genet 21:2946-60.

**POS-MON-163**

**Leptomeningeal collateral flow in experimental stroke**

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**Background:** Leptomeningeal collateral blood flow is a powerful clinical predictor of stroke size, major reperfusion and stroke outcome. Therefore collateral vessels are an extremely attractive therapeutic target for stroke. In order to trial “Collateral Therapeutics” a preclinical model with quantitative assessment of collateral flow is needed. Our aim was to develop an animal model permitting quantitative assessment of collateral blood flow during middle cerebral artery occlusion (MCAo) and reperfusion. **Methods:** Fluorescent 1 µm microspheres were visualised through a closed cranial window during MCAo in Wistar rats and reperfusion.

**Results:** Collaterals were visualised in the anterior cerebral artery (ACA) and MCA at 166 ± 85 nl/min/vessel (mean ± SE). Where the opposing flows met, spheres dived into horizontal and vertical bands of Broca were not significantly reduced (P>0.05). Counts of BF and pontine GABAergic neuron and cholinergic sectioned. Immunopositive neurons were counted in sections stained with Image J software. Individual vessel collateral flow was calculated before, during and after MCAo. **Results:** Baseline collateral flow was bidirectional (from anterior cerebral artery (ACA) and MCA) at 166 ± 85 nl/min/vessel (mean ± SE). Where the opposing flows met, spheres dived into penetrating arterioles. Immediately following MCAo, flow within the MCA reversed and dramatically increased, to 513 ± 159 nl/min/vessel at 1 min after MCAo, and peaked at 890 ± 273 nl/min/vessel at 90 minutes after occlusion. Immediately following reperfusion collateral flow was predominantly from MCA to ACA; this subsequently returned towards baseline levels at 10 min after reperfusion (257 ± 78 nl/min/vessel), and then rose again at 15 min post-reperfusion (502 ± 178 nl/min/vessel). **Conclusions:** A large but highly variable increase in flow was seen during MCAo, similar to that seen angiographically in patients. Contrary to previous reports, we found persistent blood flow through collateral vessels following reperfusion. The ability to quantify absolute blood flow will permit better modeling of the key regulators of collateral flow, and targeted investigation of potential collateral therapeutic strategies.

**POS-MON-164**

**Targeting pontine cholinergic neurons with a neurotoxic urotensin II-saporin conjugate**

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**Purpose:** In Alzheimer’s disease (AD), basal forebrain (BF) and/or pontine cholinergic neurons (CNs) are lost. These CN groups are interconnected via pontine CN projections to the BF medial septum (MS). As pontine CNs selectively express the urotensin II receptor (UIR), we tested whether saporin, a plant-derived neurotoxin, conjugated to a UIR peptide ligand (gift from Advanced Targeting Systems, San Diego CA) selectively targets pontine CNs. **Methods:** Mice (n=20, 2-4 months) anaesthetised with ketamine/xylazine (100 and 6 mg/kg) received 0.5-0.25 µg/ventricle of UIIR peptide saporin or blank saporin stereotactically injected into both ventricles. After 1-2 weeks, mice were re-anaesthetized, transcardially perfused, and their brains serially sectioned. Immunopositive neurons were counted in sections stained for CN markers (choline acetyltransferase or vesicular acetylcholine transferase, VChat). GABAergic neuron markers (parvalbumin or calbindin), or synaptophysin to mark synaptic terminals. **Results:** Mice receiving UIIR peptide-saporin had significantly reduced pontine CN counts (69% reduction in pedunculopontine tegmentum, P<0.001; 68% in laterodorsal pontine tegmentum, P<0.0001); cholinergic synaptic terminals within MS (puncta with co-localized VChat and synaptophysin) were significantly reduced in density (40%, P<0.05). BF MS CN counts were significantly reduced (46%, P=0.0001), but CN counts in the horizontal and vertical bands of Broca were not significantly reduced (P>0.05). Counts of BF and pontine GABAergic neuron and cholinergic hypoglossal motoneuron counts were not significantly reduced (P>0.05).

**Conclusions:** A novel UIIR peptide-saporin conjugate is a relatively selective neurotoxin for pontine CNs and their projections to MS. As MS CNs do not express the UIIR, their loss may reflect a reduction in anterograde neurotrophic support derived from pontine CNs.

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1Sandhoff disease1.
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POS-MON-165

SOMATOTOPIC MISMATCH OF HAND REPRESENTATION FOLLOWING STROKE - DETECTION AND FUNCTIONAL CONSEQUENCES

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Purpose: Most rehabilitation strategies to regain dexterity after stroke largely focus on methods targeting motor system; however, the function of the hand heavily depends on intact sensory input provided by tactile mechanoreceptors. Somatotopic organization is a prerequisite for meaningful interpretation of sensory input and sensorimotor control; however it is not routinely tested. The aim of the study was to design such testing method. Methods: To standardise the testing procedure, a test protocol consisting of 25 predefined stimulation points on the glabrous skin of the hand was designed. Each point was stimulated with monofilament with a force 3-5 times above detection threshold. During stimulation subjects kept the eyes closed, but then opened to indicate stimulus location by pointing on his/her hand. Subjects had also to report on various properties of the felt stimulus and whether subject was convinced about its location. Results: Normal subjects (n=6) could precisely identify the location of all test sites as did a stroke patient on a healthy hand. However, testing revealed severe distortion of somatotopic sensory maps and referred sensations on the stroke affected hand. Conclusion: While the incidence of a distorted somatotopic representation of hand area is not yet known in stroke patients, our observations indicate that in depth investigations of somatotopic organization may reveal the underlying cause of sensory deficits which would have an effect on sensorimotor control and, perhaps, central pain. Such an understanding may lead to development of new rehabilitation strategies.

POS-MON-166

CELLULAR DISTRIBUTION OF ANGIOTENSIN II RECEPTORS IN HUMAN BRAIN: RELEVANCE TO PARKINSON’S DISEASE?

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Purpose: Evidence from human and animal studies suggests that the brain renin-angiotensin system may be involved in Parkinson’s disease (PD) through angiotensin II receptor type 1 (AT1R) and type 2 (AT2R). There is little clarity, however, regarding the cellular distribution of these receptors in human brain. The principle aim of this study was to determine the cellular distribution of AT1R and AT2R in dopaminergic neurons, astrocytes and microglia in the midbrain of human post-mortem normal and PD brains. Methods: Western blotting was used to confirm the presence of AT1R and AT2R. Immunohistochemistry and immunofluorescence studies were carried out to determine the cellular distribution of the receptors (n=3 for control brains, n=3 for PD brains). As blood vessels contain both AT1R and AT2R, this was used as an internal positive control for all sections. Results: We demonstrated that AT1R was not present in dopaminergic neurons of the substantia nigra; however, it was detected in microglia of the control and PD midbrain. In contrast, AT2R was detected in dopaminergic neurons of the substantia nigra in both control and PD brains; however, only traces of AT2R were detected in microglia. Astrocytes did not express AT1R or AT2R. In frontal cortex (grey matter) both AT1R and AT2R staining was found in a subset of pyramidal neurons of the control and PD brains. Conclusion: AT1R and AT2R show differential cellular distribution in human brain; further studies are required to quantitatively analyse the expression of these receptors in different cell types in control and PD brains in order to establish their involvement in PD.

POS-MON-167

TESTING THE ‘EXTREME MALE BRAIN THEORY’

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Baron-Cohen proposed that the behaviours of Autism Spectrum Disorder (ASD) are an exaggeration of typical sex differences and proposed the ‘Extreme Male Brain Theory’, as first suggested by Hans Asperger in 1944. Almost 60 years later. Arguing that males and females empathise differently, Baron-Cohen and associates have suggested that ASD is linked with sexual dimorphism by this difference in empathy and that exposure to high levels of prenatal androgens might cause this exaggeration. Indeed, patients with ASD have higher serum androgen levels. However, neural mechanism(s) through which androgens may act to cause this difference are not understood. The aromatase knockout (ArKO, an oestrogen-deficient model) mouse model was characterised by a battery of behavioural tests. The male ArKO mice present a short-term spatial reference memory deficit in the Y-maze test; develop repetitive behaviour such as excessive water-spray triggered grooming and wheel-running activities. More importantly, the young male ArKO mice exhibited social interaction deficits (n=6, p<0.05) and ultra-sonic vocalisation deficits (p<0.05) but not the young female ArKO. These male-biased ArKO behavioural phenotypes may be analogous to the reported characteristics of ASD patients. Conclusion: Oestrogen deficiency in the brain may lead to the presentation of autistic-like symptoms predominately in male mice.

POS-MON-168

REDUCED DEPRESSIVE-LIKE SYMPTOMS IN TOLL-LIKE RECEPTOR 4 KNOCKOUT MICE

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Purpose: Depression is highly prevalent in Australia and worldwide, but available treatments only have limited efficacy. This is an indication that we do not fully understand the disease mechanisms behind depression yet. In recent years, inflammation and the development of a pro-inflammatory state have been suggested as possible contributors in the development of depression (Dantzer et al, 2008). Depressive symptoms resemble the general illness response induced by an activation of the immune system, which generates pro-inflammatory cytokines. Toll-like receptor 4 (TLR4) is an innate immune receptor important in triggering a sickness response causing a pro-inflammatory state. This pattern recognition receptor can recognise a wide range of molecules such as pathogen associated molecular patterns (PAMP) or behavioural associated molecular patterns (BAMP). The aim of this study was to demonstrate the involvement of TLR4 in depressive-like behaviour using TLR4 knockout mice. Method: Control and TLR4 knockout BALB/c mice (males, N=6-8/group) were exposed to the Forced Swim Test (FST) to measure immobility. Corticosterone levels were analysed (t=30 min) to determine the stress response and brains were collected to conduct immunohistochemistry. Results: TLR4 knockout mice show reduced immobility in the Forced swim test, a measure for depressive-like behaviour. FST induced an increase in corticosterone in control animals. Knockout animals showed a corticosterone increase at 30 min as well, but it was significantly lower than the control animals. Immunohistochemistry findings will be explained in light of the behavioural changes and reduced corticosterone response. Conclusion: It is proposed that TLR4 knockout animals show a reduced illness response and reduced depressive symptoms. Understanding the role of TLR4 in depression will open up new avenues for treatment.
AQUAPORIN MODULATORS INFLUENCE CEREBRAL ODEMA AND NEUROLOGICAL OUTCOME FOLLOWING TRAUMATIC BRAIN INJURY

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Purpose: Despite advances in critical care management, traumatic brain injury (TBI) continues to be a leading cause of death and disability in peptides. Pathological swelling (cerebral oedema) arising from such injuries is of major significance to patient clinical outcomes, yet treatment approaches remain largely symptomatic. Recent experimental findings have implicated aquaporin (AQP) water channels in the formation and resolution of cerebral swelling, with particular emphasis on AQP4, which is localised to astrocytes and ependymal cells. This study therefore used specific modulators of AQP4 channels to ascertain effects on cerebral oedema following TBI. Methods: Adult male Sprague-Dawley rats (250-280 g) were injured using the impact acceleration model of TBI and administered either an AQP4 agonist (AgF026, 0.2 mg/kg iv), an antagonist (AqpB013, 0.8/mg/kg iv) or equal volume DMSO vehicle at 30min post injury. At 5h following trauma, animals were assessed (n=5-6/group) for cerebral oedema via the wet-weight/dry-weight methodology, and histologically using haematoxylin and eosin. Albumin and amyloid precursor protein. A subgroup of animals was assessed at 7d for functional outcome using the accelerating rotorad motor task (n=6/group).

Results: Administration of an AQP4 antagonist significantly attenuated cerebral oedema at 5h and improved functional outcome at 7d. Albumin leakage and cell injury were also ameliorated in antagonist treated animals compared to the other injury groups. In contrast, administration of an AQP4 agonist exacerbated cell injury, albumin leakage and functional deficits despite reducing brain swelling, albeit to a lesser extent than antagonist treated animals. Conclusion: Our findings suggest that an AQP4 antagonist administered early after diffuse TBI attenuates oedema formation and improves functional outcome.

THE THERAPEUTIC EFFECTS OF AN OLEANOLIC ACID DERIVATIVE IN DIET INDUCED OBESITY

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Purpose: Recently, studies have shown that pentacyclic triterpenes have anti-inflammatory effects in the brains of mice with obesity; a disorder where inflammation largely contributes to energy balance dysregulation. The purpose of this study was to: 1) Investigate for the first time if the oleanolic acid (OA) derivative, barboxolone methyl (BM) has a more potent effect than ursolic acid (UA) and OA in reducing body weight in mice fed a high fat diet (HFD) for 10 weeks; 2) To analyse hypothalamic tissue of mice in this study to determine if UA, OA and BM improve key neuronal molecules involved in energy expenditure. Methods: 12 week C57Bl/6 male mice (n=70) were divided into 5 groups (low fat diet (LFD), HFD; HFD + UA, HFD + OA and HFD +BM). BM, UA and OA were administered in drinking water at a dosage of 10mg/kg. Signalling molecules involved in energy expenditure is being analysed using western blotting and Real time polymerase chain reaction (RT-PCR). One and two way analysis of variance (ANOVA) and post-hoc Tukey-HSD test were performed for statistical analysis. Results: BM significantly prevented body weight gain in mice fed a HFD compared to the mice treated with OA and UA (p≤<0.05). Furthermore, BM reduced body weight (p<0.05). No differences in energy and water intake among the HFD groups have been found (p>0.05). The experiment is currently in progress and further data will be presented at the conference.

Conclusion: The preliminary data have shown that BM significantly reduces body weight gain in mice fed a HFD (p≤<0.05). These results indicate that BM induced negative energy balance is influenced by the energy expenditure pathway of neuronal networks.

A NOVEL T-TYPE CALCIUM CHANNEL ANTAGONIST DELAYS THE PROGRESSION OF EPILEPTOGENESIS IN THE AMYGDALA KINDLING MODEL OF TEMPORAL LOBE EPILEPSY

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Purpose: Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults that is refractory to medical treatment. Current therapeutic treatment is symptomatic, suppressing seizures, but has no disease modifying effect on epileptogenesis. T-type Ca2+ channels have been implicated in pathogenesis of limbic epileptogenesis, therefore the current study set out to investigate the effects of a novel T-type Ca2+ channel antagonist (Z944, Zalicus Pharmaceuticals) on the progression of epileptogenesis in the amygdala kindling model of TLE. We have previously shown that Z944 was not effective at suppressing seizures of amygdala kindled rats to reach a fully kindled state than animals receiving ETX or vehicle. Z944 required more stimulations to evoke a class III, IV or V seizure and to reach a fully kindled state than animals receiving ETX or vehicle.

Results: Animals receiving Z944 required more stimulations to evoke a class III, IV or V seizure and to reach a fully kindled state than animals receiving ETX or vehicle. Z944 required more stimulations to evoke a class III, IV or V seizure and to reach a fully kindled state than animals receiving ETX or vehicle. Z944 required more stimulations to evoke a class III, IV or V seizure and to reach a fully kindled state than animals receiving ETX or vehicle.

Conclusion: Our findings suggest that an AQP4 antagonist administered early after diffuse TBI attenuates oedema formation and improves functional outcome.

POSTERS MONDAY

INVESTIGATION OF THE ROLE OF BMP RECEPTOR IA IN OLIGODENDROCYTE PROGENITOR CELLS IN VITRO AND DURING DEMYLINATION

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Oligodendrocyte cell death is a key pathological event in demyelinating diseases and leads to axons losing their myelin sheaths, which results in disruption to axon conduction. Enhancement of oligodendrocyte regeneration by endogenous progenitor cells (OPCs) is a promising strategy for repair. We have shown that Bone Morphogenic Protein (BMP) signaling is increased in myelin lesions in mice and that inhibition of BMP4 increases new oligodendrocytes during myelin repair. BMP4 signals through two type I signal transducing receptors (BMPRIa and BMPRIRb). Based on our data and the knowledge that BMPRIa is highly expressed in OPCs, we hypothesize that BMPRIa in OPCs may play an important role in oligodendrocyte differentiation and myelin pathology. Methods: We created a new transgenic mouse line with inducible, conditional knockout of BMPRIa in OPCs. We are using primary cultures of cortical OPCs from these mice to assess the effects of BMPRIa cKO in vitro and cuprizone-induced demyelination to assess in vivo effects of BMPRIa cKO on myelin pathology. Results: We confirmed Tamoxifen-induced recombination in brains in our mouse line in vivo (n=6,6 animals; p<0.001) and in cultures of cortical OPCs (n=5,5 independent cultures; p<0.001). We are currently assessing the results of experiments utilizing cuprizone-induced demyelination in littermates with and without tamoxifen-induced BMPRIa cKO and in differentiation assays in cortical OPC cultures from these mice. Conclusions: Results from our experiments will help inform us as to whether BMPRIa in OPCs plays a role in experimental myelin pathology and if disruption to BMP signaling by BMPRIa cKO increases myelin repair, and therefore, constitutes a potential therapeutic target.
RESPONSE OF HUMAN SPINAL CORD EPENDYMAL CELLS TO TRAUMATIC INJURY

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Purpose: Animal models have demonstrated that endogenous neural progenitor cells located in the sub-ependymal region of the spinal cord respond to traumatic injury. These cells have the potential to be harnessed as a treatment for spinal cord injury but relatively little is known about their response in human spinal tissue. Methods: Cervical spinal cord tissue was examined from 41 cases where cause of death was due to traumatic injury or other causes (controls) including embryonic (n=2), infant (n=10), child (n=17) and adult (n=12). Anti-Nestin and Anti-GFAP immunohistochemistry were used to examine cellular responses to traumatic injury. Results: There were no significant age related differences in the size or shape of the central canal other than younger spinal cords have a pseudostratified epithelium compared to the simple columnar epithelium seen in adults. Nestin positive immunoreactivity (IR) was prominent in a sub-set of ependymal cells with long basal processes located in the ventral and dorsal aspects of the central canal. The percentage of these nestin positive ependymal cells was increased in cases of traumatic injury compared to controls (Mann Whitney, p < 0.05). Endogenous neural progenitor cells were unable to be positively identified using anti-Nestin IR alone. Conclusion: Nestin positive ependymal cells in the human central canal are increased following traumatic injury to the central nervous system.

A TRUNCATED FRAGMENT OF SRC PROTEIN KINASE GENERATED BY CALPAIN-MEDIATED CLEAVAGE IS A MEDIATOR OF NEURONAL DEATH IN EXCITOTOXICITY

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Excitotoxicity resulting from over-stimulation of glutamate receptors is a major cause of neuronal death in cerebral ischemic stroke. The over-stimulated ionotopic glutamate receptors exert their neurotoxic effects by over-activation of calcium inclusions which trigger neuronal death by catalysing limited proteolysis of specific cellular proteins. Purpose: To define the role of the protein tyrosine kinase Src in neuronal survival and excitotoxic neuronal death. Methods: Cultured mouse primary cortical neurons treated with glutamate or transduced with lentiviral vectors directing the expressing recombinant Src and its mutants were used in our study. The findings made in the cultured neurons are confirmed by investigation in a rat model of ischemic stroke. Results: Here, we report that in cultured cortical neurons and in vivo in a rat model of focal ischemic stroke, the tyrosine kinase Src is cleaved by calpain at a site in the N-terminal unique domain. This generates a truncated Src fragment of approximately 52 kDa, which we localized predominantly to the cytosol. A cell membrane- permeable fusion peptide derived from the unique domain of Src effectively prevents calpain from cleaving Src in neurons and protects against neuronal death induced by glutamate over-stimulation. To explore the role of the truncated Src fragment in neuronal death, we studied the effect of lentivirus-directed expression of a recombinant truncated Src fragment on survival of cultured neurons. Expression of this fragment, which lacks the myristoylation motif and unique domain, was sufficient to induce neuronal death. Furthermore, the kinase activity is indispensable to its neurotoxic action. Conclusion: As Src maintains neuronal survival, our results implicate calpain cleavage as a molecular switch converting Src from a promoter of cell survival to a mediator of neuronal death in excitotoxicity. Besides unveiling a new function of Src, our discovery of the neurotoxic action of the truncated Src fragment suggests new therapeutic strategies with potential to minimize brain damage in ischemic stroke.

ESTABLISHING THE ROLE OF SEROTONERGIC NEURONS IN DYSKINESIAS IN PARKINSONS DISEASE

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Purpose: Dyskinesias are abnormal, involuntary movements that occur as a consequence of levodopa treatment in Parkinson’s disease (PD), and represent a substantial barrier to effective symptomatic management. Animal models implicate serotonin neurons in the pathophysiology of dyskinesias, but human studies are lacking. We aimed to test this hypothesis in post mortem brain tissue from PD patients. Methods: We collected brain tissue from brain banks in Melbourne, Sydney and London according to specific inclusion and exclusion criteria. We used HPLC to measure levels of serotonin, dopamine and their metabolites in caudate (HPLC, p<0.001) compared to controls, but there was no correlation between serotonin function and dyskinesia severity. This may explain why treatment of these debilitating drug-induced side effects has remained elusive.

EFFECTS OF AN ENRICHED ENVIRONMENT ON MOTOR FUNCTION FOLLOWING ISCHEMIC STROKE

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Purpose: Enriching housing for rodents has been shown to dramatically facilitate recovery from traumatic brain injury. In the current study we assessed the ability of an enriched environment to enhance recovery of complex motor function in the rat following ischemic stroke. Methods: Adult male Long Evans rats (n=20) underwent 90 or 150 minute middle cerebral artery occlusion. Half of the animals in the 90 and 150 minute groups were placed in standard housing following stroke induction, whereas the others were placed in a multilevel enriched environment. Sham treatment groups were also included. At 1 and 3 months post-stroke animals underwent a battery of motor tests, including removal of a sticky dot from the forepaw and rotating beam traversal. Results: Our results indicated that a 90 minute occlusion produced profound weight loss that persisted over several weeks, an effect that was dramatically exaggerated in the animals that underwent a 150 minute occlusion. No clear differences were observed between animals housed in an enriched or non-enriched environment. The sticky dot test was extremely inaccurate in identifying neurological deficits in post-stroke animals. However, animals in the 150 minute occlusion group had significantly more problems successfully traversing the rotating beam. Conclusion: We believe that the inability to detect a difference between enriched and non-enriched animals was largely due to the amount of intense experimenter-animal contact required to ensure recovery following stroke. In conclusion, the thread occlusion model may not be the most appropriate method to induce stroke in rats to examine recovery.
**POS-MON-177**

**AXONAL PATHOLOGY AND NMJ DEGENERATION IN THE YFP X MSOD1G93A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

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**Purpose:** Recent investigations indicate that axonal dysfunction, prior to cell loss, may be a causative factor of the initial symptoms of Amyotrophic lateral sclerosis (ALS) and that distal degeneration may occur before the onset of disease symptoms. The current study utilised mSOD1<sup>G93A</sup> mice crossed with YFP (yellow fluorescent protein, Thy-1 promoter) to investigate the mechanisms underlying the development of axonal and neuromuscular junction (NMJ) pathology in ALS. **Methods:** mSOD1<sup>G93A</sup> YFP+ve mice and wild-type controls (n=2 for each group) were perfused at 8 and 20 weeks of age, the gastrocnemius muscle cryosectioned (80μm) and labeled with βBungarotoxin-594 (βBI). To investigate NMJ degeneration βBI-YFP colocalisation, axonal and NMJ morphology and NMJ diameters were compared. **Results:** There was no significant difference in the number of intact axons (6.70 ± 1.67) and NMJs (5.95 ± 1.10) at 8 weeks in mSOD1<sup>G93A</sup> mice in comparison to control (6.62 ± 2.76, 6.82 ± 1.17 axon and NMJ respectively). However, there was a significant (p<0.05) increase in the number of intact MNJs (1.31 ± 0.42) with incomplete synaptic coverage from 8 to 20 weeks in mSOD1<sup>G93A</sup> mice (0.77 ± 0.31) in comparison to control (0.17 ± 0.13). By 20 weeks, the gastrocnemius muscle of the mSOD1<sup>G93A</sup> mice had significantly (p<0.05) less intact axons (2.25 ± 0.92) and NMJs (1.99 ± 0.85) in comparison to 8 week mSOD1<sup>G93A</sup> mice. However, there was no significant difference in the mean number of synapses with incomplete synaptic coverage from 8 to 20 weeks (2.15 ± 0.84, p<0.001) in mSOD1<sup>G93A</sup> mice. **Conclusion:** Axonal pathology and changes to NMJ functionality, morphology and size are characteristic of ALS. Using a YFP transgenic mouse strain, crossed with the established mSOD1<sup>G93A</sup> it is possible to examine these changes over time and uniquely in real-time to identify pathological process, morphological changes and underlying disease mechanisms.

**POS-MON-177**

**A ROLE FOR INTERNEURONS IN ALS?**

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**Purpose:** Hypereexcitability has been implicated in the pathogenesis of Amyotrophic Lateral Sclerosis and it has been proposed that this may be due to alterations in the influence of interneuron populations. The present study aimed to investigate cortical inhibitory influence in the G93A-SOD1 mouse model of ALS. Immunohistochemistry was performed to identify qualitative and quantitative lamina specific pathological changes to interneuron subpopulations as identified by calcium binding and neuropeptide protein markers. **Method:** G93A-SOD1 (end-stage, n=4) and wild-type control (20 weeks, n=4) animals were transcardially perfused with paraformaldehyde and brains serially cryosectioned (40μm). Immunohistochemistry and confocal analysis was performed using standard protocols. **Results:** High power analysis of motor cortex found the calcium binding protein calretinin (CR) had irregular labeling of cell soma in lamina II/III, with fewer typically ovoid cells present. Analysis of G93A-SOD1 and wild type cortical regions found the number of CR immunopositive interneurons in the largely supragranular lamina of the primary motor cortex (I-IV) to be significantly decreased (P<0.05) by 37% in G93A-SOD1 mice (n=4, 12.53 ± 2.137 SEM), as compared with wild type control (n=4, 19.59 ± 2.452 SEM) (Two-way ANOVA, multiple comparisons Bonferroni Test), whereas labeling for parvalbumin, identified no significant difference. Analysis of neuropeptide Y (NPY) expression identified a significant increase (P<0.05) in labeled cells in the infragranular motor cortex (V-VI) in G93A-SOD1 mice (11 ± 1.493 SEM), as compared with wild type controls (6.592 ± 0.879 SEM). **Conclusion:** This investigation demonstrates decreases in GABAergic inhibition, with specific interneuron populations and functional domains of cortical inhibitory processing affected, thus providing an alternate mechanism for motor neuron vulnerability and excitotoxicity in ALS.

**POS-MON-179**

**RESPONSES TO FOCAL BRAIN INJURY IN APP/PS1 MICE**

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**Purpose:** Epidemiological studies have demonstrated brain injury as a risk factor for the development of Alzheimer’s disease. The purpose of this study was to optimise a mouse model of focal brain injury to examine axonal injury responses in the setting of amyloidosis. Nine-month old APP/PS1 mice were used as a model of amyloidosis as they exhibit numerous injury responses in the setting of amyloidosis. Nine-month old APP/PS1 mice were used as a model of amyloidosis as they exhibit numerous injury responses in the setting of amyloidosis. Three-month old C57/Bl6 and 9-month old APP/PS1 and C57/Bl6 male mice underwent injury and were sacrificed 48 minutes. The heat generated by drilling into the skull caused microglial activation and axonal pathology in underlying brain tissue. Thus, periodic drilling with a 4°C drill bit and irrigation with 4°C PBS was used, preventing this effect. Microglia and axons were immunolabelled with the markers β-2-Microglobulin and parvalbumin, identified no significant difference. Analysis of neuropeptide Y (NPY) expression identified a significant increase (P<0.05) in labeled cells present. Analysis of G93A-SOD1 and wild type cortical regions found the number of CR immunopositive interneurons in the largely supragranular lamina of the primary motor cortex (I-IV) to be significantly decreased (P<0.05) by 37% in G93A-SOD1 mice (n=4, 12.53 ± 2.137 SEM), as compared with wild type control (n=4, 19.59 ± 2.452 SEM) (Two-way ANOVA, multiple comparisons Bonferroni Test), whereas labeling for parvalbumin, identified no significant difference. Analysis of neuropeptide Y (NPY) expression identified a significant increase (P<0.05) in labeled cells in the infragranular motor cortex (V-VI) in G93A-SOD1 mice (11 ± 1.493 SEM), as compared with wild type controls (6.592 ± 0.879 SEM). **Conclusion:** This investigation demonstrates decreases in GABAergic inhibition, with specific interneuron populations and functional domains of cortical inhibitory processing affected, thus providing an alternate mechanism for motor neuron vulnerability and excitotoxicity in ALS.

**POS-MON-180**

**LOLITREM B INTOXICATION ACTIVATES NEURONAL STRESS PATHWAYS**

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**Purpose:** Lolitrem B is an indole–diterpenoid alkaloid toxin found primarily in perennial ryegrass. It is a potent inhibitor of large conductance Ca<sup>2+</sup> activated potassium (BK) channels. Ingestion of toxin causing cerebellar ataxia, tremor and Purkinje cell lesions has been extensively reported in grazing livestock. However, it has been suggested that a more widespread syndrome exists which includes anxiety, hypoaesthesia, allodynia and cognitive dysfunction. This study used behavioural testing to assess cognitive function and c-Fos immunohistochemistry to determine the level of activation in neuronal stress pathways following acute lolitrem B exposure in the mouse. **Methods:** Adult mice were injected with lolitrem B (2mg/kg, IP) (n=12) or vehicle (DMSO) (n=12). Tremor was analysed using a pressure sensor and ADI PowerLab<sup>®</sup> analytical. Spatial memory and learning, object recognition, and motor function were tested using the AnyMaze<sup>®</sup> system. Additionaly, at three hours post injection four mice from each group were anaesthetised and transcardially perfused with 4% paraformaldehyde. Brains were sectioned at 40μm and c-Fos immunoreactivity was revealed using the avidian-biotin-horseradish peroxidase technique. **Results:** Analysis confirmed tremor in the range of 11-17Hz at one hour and increasing to 18-25Hz by three hours, peaking at nine hours. A single dose of lolitrem B did not induce spatial learning or memory deficits despite appearing to induce short term abnormalities in normal exploratory behaviour. Counts of c-Fos-IR nuclei revealed a significant increase in the nucleus tractus solitarius, ventromedial medulla, parabrachial nucleus, central amygdala, and paraventricular hypothalamus following acute lolitrem exposure. No c-Fos-IR was detected within the cerebellum. **Conclusion:** Lolitrem B induced a pattern of c-Fos immunoreactivity that is consistent with previous clinical and experimental observations of intoxication inducing anxiety, allodynia and hyperaesthesia in production livestock.
POS-MON-181

DELINEATING THE NEUROPROTECTIVE REGION WITHIN THE AMYLOID PRECURSOR PROTEIN FOLLOWING TRAUMATIC BRAIN INJURY

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Purpose: Traumatic brain injury (TBI) is a significant health problem and is the leading cause of death in Australians under the age of 45. Despite this there are no effective treatments to limit the ongoing cell death, with suggestions that new therapeutics could aim to emulate the body's own neuroprotective pathways. One such agent is the amyloid precursor protein (APP), as knockdown of APP has been shown to exacerbate motor and cognitive deficits following TBI, with an associated increase in neuronal cell death. This relates to lack of sAPPα, the product of non-amyloidogenic processing of the APP, as treatment with exogenously added sAPPα mice improves histological and functional outcome such that APP-/- mice were no longer significantly different to wildtype mice. However the exact regions within sAPPα which confer this neuroprotective activity are yet to be determined, with earlier research suggesting it may relate to the heparin binding sites. Methods: To further investigate the specific regions encompassing the two known heparin binding sites within APP (APP96-110 and APP472-503) were given via intracerebroventricular injection to APP-/- mice following controlled cortical impact TBI. Results: Treatment with APP96-110 significantly improved motor and cognitive outcome in these mice (p<0.05), as detected on the ledged beam and Barnes Maze respectively, with an associated significant decrease in neuronal cell death within the cortex and hippocampus (p<0.05). In contrast APP472-503 had no effect on functional or histological outcome. Conclusion: This suggests that the neuroprotective actions of sAPPα relate to the heparin binding site located within the region APP96-110, with further studies needed to determine whether this could be used as a potential therapeutic agent following TBI in wildtype animals.

POS-MON-183

SRY IS LOCALIZED IN MIDBRAIN DOPAMINE NEURONS AND IS HIGHLY RESPONSIVE TO 6-HYDROXYDOPAMINE

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Purpose: Parkinson’s disease affects more men than women. This sex bias may be attributed to XY chromosome effects. The male sex is determined through the action of the sex determining region on the Y chromosome (SRY) transcription factor. The unexpected action of SRY in the human midbrain and function in vitro. SRY immunoreactivity was detected in the human male midbrain samples (n=5), but not female (n=2), substantia nigra pars compacta within a sub-population of tyrosine hydroxylase (TH)-positive neurons. SRY protein also co-localised with TH-positive neurons in the VTA, GAD-positive neurons in the SNc and calbindin-positive neurons of the dorsal SNc. In the human XY neuroblastoma line BE(2)-M17, SRY was found to regulate the transcription of genes which control dopamine synthesis, metabolism and signalling; TH, DDC, DBH, MAO-A and D2R. Overexpression of SRY led to increased extracellular dopamine. To examine whether SRY expression might be altered within a diseased dopamine neuron, we tested the response of SRY to stress induced by toxin models of Parkinson’s disease. SRY was highly up-regulated (9-fold) by 6-hydroxydopamine at 40 µM compared to vehicle and more mildly (2-3-fold) by para-quinone and dopamine itself. There was no effect of SRY with hydrogen peroxide treatment. A 2.2 kb fragment of the SRY promoter region was activated in cells treated with all toxins except hydrogen peroxide. (All n=3, p<0.05).

Results:

Conclusions: Combined, these results suggest that SRY may play a role as a positive regulator of catecholamine biosynthesis in a healthy XY midbrain dopamine neuron and that misregulation of SRY in a diseased state might alter the normal function of the gene and exacerbate cell death, contributing to the male bias in Parkinson’s disease.

POS-MON-182

THE STUDY OF HINDBRAIN STROKE WITH A MOUSE MODEL OF PHOTOTHROMBOSIS-INDUCED ISCHAEMIA

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Purpose: The mortality rate following a cerebellar infarction is 23% greater than an infarct in other brain regions. Moreover, vertebral-basilar stroke has a mortality rate of 85%. Despite the high mortality and morbidity of hindbrain strokes, there are limited animal models to aid research. We therefore investigated whether photothrombosis may provide the necessary spatio-temporal control of infarct volume; further we resolved to settle the controversy over whether this technique provides a thrombotic infarct or, as recently suggested, the injury is independent of platelet aggregation-mediated occlusion. Methods: Photothrombosis was achieved by intravenous administration of rose bengal followed by a brief illumination at 561nm of the region of interest within the cerebellum. The clot formation was visualised in real-time by intravitral multi-photon imaging of the platelet aggregation (using Cy7-labelled anti-CD42). Infarcts were examined by high-field (9.4T) T2 weighted MRI at days 1, 4, and 7 post-ischaemia. Tissue was subsequently paraffin embedded, sectioned, and stained with haematoxylin and eosin for histological verification of the infarct and surrounding regions. Results: The MRI scans showed acute infarction of ~1mm^3 that corresponded to the primary vascular occlusion. The anti-CD42 platelet marker showed the clot was restricted to the same area. The T1 and T2 relaxation times were prolonged due to altered diffusion and histology consistent with a penumbra. The control study delivered the equivalent photonic flux without the rose bengal infusion. This brain tissue lacked platelet aggregation and an infarct. Conclusion: Here we show that photothrombosis can induce hindbrain ischaemia, based on real-time imaging of thrombus development, MRI of the progression of these cerebellar infarcts, and histological analysis of the thrombus in the brain tissue.

POS-MON-184

CHARACTERISING A315T TDP-43 TRANSGENIC MICE AS A MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL LOBAR DEGENERATION

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Purpose: TAR DNA-binding protein-43 (TDP-43) is involved in RNA processing pathways. In amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), TDP-43 is aberrantly metabolised and accumulates in cytoplasmic inclusions. The mechanisms by which TDP-43 contributes to these diseases are unclear. Transgenic mice that over-express TDP-43 containing the disease-causing A315T mutation represent a potential new animal model for ALS and to recapitulate aspects of TDP-43 pathology. The aim of this study was to further characterise this model. Methods: Male transgenic A315T TDP-43 mice (n=15) and non-transgenic littersmates (n=13) were assessed for motor and cognitive function using the Rotarod and Y-maze tests. Brain and spinal cord samples were collected at late disease stage and western blot analysis performed for markers of neuronal function and health including TDP-43, survival motor neuron, vascular endothelial growth factor (VEGF), ERK, Akt, glycogen synthase kinase 3 and histone deacetylase 6. Results: A315T TDP-43 mice displayed a progressive decline in locomotor function. A decrease in cognitive function could not be detected. Western blot analysis confirmed over-expression of the A315T TDP-43 protein in spinal cord and brain of transgenic mice. A significant increase was also detected in VEGF expression of transgenic mice but only in the spinal cord. Expression of other markers was not altered in the transgenic mice. Conclusion: The progressive loss of motor function in A315T TDP-43 transgenic mice is consistent with symptoms of ALS. Changes in VEGF are consistent with other mouse models of the disease. Collectively these results indicate A315T TDP-43 mice recapitulate some aspects of ALS.
POS-MON-185

EFFECTS OF CHRONIC ANTIPSYCHOTIC TREATMENT ON DOPAMINE SYNTHESIS

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Purpose: The mesolimbic dopamine (DA) pathway, with projections from the Ventral Tegmental Area (VTA) to the Nucleus Accumbens (NAC), is involved in antipsychotic drug (APD) treatment for schizophrenia symptoms. Whilst APD mechanisms of action have been thought through DA D2-receptor antagonism, recent developments of D2 partial-agonist APDs has thrown to light the potential of controlling DA synthesis. This study aimed to investigate the effects of APDs Haloperidol (a D2 antagonist) and Aripiprazole (a D2 partial agonist), on DA synthesis markers in the mesolimbic DA-pathway.

Methods: Male Sprague-Dawley rats (n=6/group) were administered orally with Aripiprazole (0.75mg/kg, t.i.d.), Haloperidol (0.1mg/kg, t.i.d.), or vehicle (control) for 10-weeks. Levels of Phospho-Tyrosine Hydroxylase (p-TH), Tyrosine Hydroxylase (TH), and Dopamine Transporter (DAT) was determined by Western blot, whilst DOPA and DA levels were measured by Gas Chromatography-Mass Spectrometry.

Results: In the VTA, Aripiprazole and Haloperidol decreased p-TH and TH expression. In the NAC, both treatments decreased expression (both p<0.01). In addition, Aripiprazole decreased DA levels (p<0.05), and Haloperidol treatment decreased DOPA levels (p<0.05) compared with controls. Haloperidol significantly increased DAT level in the NAC (p<0.05). Conclusion: Although both Aripiprazole and Haloperidol have well documented mechanisms of action on DA receptors to provide their therapeutic efficacy, this study revealed that they also have significant effects on DA synthesis in the mesolimbic pathway and in turn a low DA availability in the synapse. Future studies into the pharmacology of APDs may look into their exact mechanisms of action on the regulation of DA synthesis; potentially providing further knowledge behind Aripiprazoles therapeutic efficacy and future potential APD development.

POSTERS}

POS-MON-186

BINDING AFFINITY OF ARIPIPRAZOLE TO THE DOPAMINE D2 RECEPTOR IN DIFFERENT REGIONS OF THE RAT BRAIN

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Purpose: The new antipsychotic drug aripiprazole has a high affinity for the dopamine D2 receptor (D2R) and can treat multiple symptoms of schizophrenia with reduced risk of extrapyramidal side effects (EPS). The question of how aripiprazole achieves its therapeutic benefits through the D2R, without causing EPS is currently unanswered. This study aimed to examine the binding affinity of aripiprazole to D2R in specific nuclei of the mesolimbic and nigrostriatal dopamine pathways, which are implicated in the therapeutic effects and EPS side effects of antipsychotic drugs, respectively. METHODS: Brain tissue obtained from male Sprague Dawley rats (n=7) was sectioned onto polylyline slides and D2Rs were visualised by competition autoradiographic techniques using 20nM [3H]raclopride. D2R binding density was quantified in the ventral tegmental area (VTA) and nucleus accumbens (NAC) (mesolimbic pathway), substantia nigra (SN) and caudate putamen (CPU) (nigrostriatal pathway). The average results of 7 animals were combined for each region of interest. RESULTS: Aripiprazole had a high affinity for the D2R in all regions examined. Aripiprazole had mean inhibition constants (Ki) of: 15nM in the VTA, 71nM in the NAC, 21nM in the SN, 29nM in the CPU. CONCLUSION: Aripiprazole has a high binding affinity for the D2R in specific nuclei of the dopaminergic pathways in the rat brain. Aripiprazole affinity for D2Rs in the NAC was slightly lower than the other regions of interest; however values were similar in range, therefore differences in binding affinity cannot explain its reduced EPS. The mechanisms by which aripiprazole achieves its therapeutic effects, while avoiding EPS, require further investigation.

POS-MON-187

DECREASED NMDA RECEPTOR NR1 SUBUNIT IN POST-SYNAPTIC DENSITY FROM PREFRONTAL CORTEX OF PATIENTS WITH SCHIZOPHRENIA

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Purpose Our group has recently observed decreased mRNA and protein expression of the obligatory NR1 subunit of the N-Methyl-D-Aspartate receptor (NMDAR) in the dorsolateral prefrontal cortex of patients with schizophrenia (Weickert et al., Molecular Psychiatry, doi:10.1038/mp.2012.137). Following on from this observation, we asked whether NR1 protein levels in the post-synaptic density (PSD) from the prefrontal cortex (PFC) differs between schizophrenia patients and controls. Method Postmortem human brain tissue from the frontal pole region of the PFC from schizophrenia patients (n=37) and control (n=37) subjects was obtained from the New South Wales Tissue Resource Centre. Groups were matched on age, gender, tissue pH, and postmortem interval. Subcellular tissue fractionation was carried out to isolate the PSD fraction. Western blotting was used to quantify the amount of NR1 subunit protein in the PSD fractions relative to α-tubulin. Results Our data indicate that the schizophrenia patient cohort had a significantly lower amount of NR1 protein in the PSD fraction compared to the control group ([t(66)=2.293, p=0.025, two-tailed Student’s t-test). Conclusion The lower levels of NMDAR NR1 protein in the PSD from schizophrenia patients in the context of lower expression of total NR1 mRNA and protein is consistent with a deficit at the level of NR1 synthesis. An alternate interpretation is that few NMDARs at the PSD leads to reduced synthesis of the NR1 subunit and/or may indicate lower levels of trafficking of NMDA receptor complexes to the PSD.

POS-MON-188

TUMOUR NECROSIS FACTOR-ALPHA (TNFα) DISRUPTS THE CELL CYCLE OF HUMAN NEURAL STEM/PROGENITOR CELLS: MODELING INFLAMMATORY PATHOLOGY IN HUMAN NEUROGENESIS AND NEURODEVELOPMENTAL DISORDERS

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Purpose: Environmental factors contribute to the etiology of neurodevelopmental disorders. For example, a variety of maternal infections have been implicated in schizophrenia, bipolar disorder, and autism. Inflammation caused by infection and involving cytokines produced by the mother, placenta, or fetus represents a common mechanism, potentially altering pre- and perinatal brain development. Despite the evidence for a role of cytokines in development and disturbance in the link between maternal infection and disease pathology remains unclear. Here we report using a human model system that the pro-inflammatory cytokine TNFα disrupts neural stem/progenitor cell (NSPC) cycling. Methods: Human NSPCs (hippocampal: n=2; subventricular zone: n=2) were derived from normal term newborns and treated with 100U/ml TNFα for 48 hours and cell cycle was assessed by flow cytometry of proliferation markers, and propidium iodide for DNA content. Results: Untreated NSPCs stably expressed sox-2, vimentin, nestin and Ki-67 (>90%). TNFα treatment decreased the number of cells displaying total Ki-67 staining, indicative of G2/M phase (p<0.05). DNA content analysis showed no change in the proportion of 4N cells, while the proportion of 2N cells increased and S-phase cells decreased. Conclusion: Our findings suggest that TNFα disrupts the cell cycle of human NSPCs, and provide new insight into how inflammation arising from infection may contribute to aberrant neurogenesis in disorders of brain development.
POS-MON-189

A RAPID METHOD TO QUANTIFY NEUROGENESIS USING IMMUNOBLOTTING AND THE EFFECT OF PARKINSON’S DISEASE ON NEUROGENESIS IN THE SUBVENTRICAL ZONE


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Purpose: Significant changes in neurogenesis have been reported in a variety of neurodegenerative diseases, including a recent finding from our laboratories of reduced neurogenesis in Parkinson’s disease (PD). Classic methods to quantify neurogenesis involve identification of proliferating and migrating cells using a variety of immunocytochemical markers and quantification at the single cell level. Such methods are time- and labour-intensive. Here we investigated immunoblotting as a rapid method to quantify different stages of neurogenesis in the normal and parkinsonian subventricular zone (SVZ). The data were compared with our previous findings in the PD SVZ using a classic immunohistochemical approach. Methods: Immunoblots were performed on fresh frozen SVZ tissue from 5 control and 10 PD brains for the following markers: glial fibrillary acidic protein-delta (GFAP-delta; stem cell marker), proliferating cell nuclear antigen (PCNA; proliferation marker) and neuronal class III B-Tubulin (TUJ1; migrating neuroblast marker). Results: Consistent with our previous finding of reduced numbers of migrating neurons in the PD SVZ, levels of GFAP-delta and PCNA protein in the SVZ did not differ between control and PD brains but levels of TUJ1 protein was significantly reduced (by 63%) in the PD SVZ, compared with controls. Conclusion: These data confirm our previous finding of a significant decrease in number of early migrating neurons in the parkinsonian SVZ. Further they suggest that immunoblotting of neurogenesis marker proteins represents a rapid method to quantify different stages of neurogenesis in fresh human brain.

POS-MON-190

EDU LABELLING FOR HISTOLOGICAL AND MOLECULAR PROFILING OF PROLIFERATING CELLS IN BRAIN TUMOURS

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Proliferation is an important cellular process in cancer progression and is a primary target for cancer research and drug discoveries. Consequently, many therapeutic strategies target proliferating cells yet the field lacks effective techniques to separate the population for drug target discovery. Here, 5-Ethynyl-2′-deoxyuridine (EdU) allows proliferative cell labelling in glioma mouse models allowing characterisation of proliferating and non-proliferating cells within the tumour parenchyma. Using our established tumour models in mice and employing the combined techniques of click chemistry, immunofluorescence (IF), fluorescence in-situ hybridization (FISH), fluorescence activated cell sorting (FACS) and quantitative real time PCR, we characterize EdU-treated human glioma in-vivo. Image analysis showed extensive labelling of proliferating tumour cells and mouse brain cells localising EdU, allowing characterisation of cellular constituents within the tumour parenchyma: proliferating tumour cells (46.0%±8.29%), non-proliferating tumour cells (38.19%±4.79%), proliferating mouse cells (5.89%±0.89), and non-proliferating mouse cells (9.82%±3.73). We also demonstrate that EdU is a powerful tool to identify proliferating cells, allowing their isolation for molecular profiling. We show for the first time, the separation of actively proliferating cells and non-proliferating cells from tumours in-vivo using FACS, for their molecular profiling. The average RNA yields for proliferating and non-proliferating cells were 99.60ng/µL and 93.37ng/µL respectively with the A260/280 absorbance ratio measuring 2.0, providing sufficient RNA quantity and quality for gene expression profiling. This study demonstrates the robustness of using EdU in characterising actively dividing tumour cells, forming the basis for protein and gene expression profiling of proliferative and non-proliferative cells in a preclinical model of brain tumour.

POS-MON-191

METABOLOMICS AS A TOOL TO INVESTIGATE α1-ADRENERGIC RECEPTOR-MEDIATED SIGNALING IN CORTICAL NEURONS

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Purpose: Complex regional pain syndrome (CRPS) is a neuropathic pain condition that can be acquired after minor trauma or surgery to soft tissues and nerves. Biopsies from affected tissue show an increased density of α1-adrenergic receptors (AR1) compared to controls. In order to further study the role of AR1 in CRPS, we wish to use an immortalized cortical neuron cell line, NIE115. The present study aimed to (i) characterise the expression of AR1 on NIE115 cells and (ii) document how the metabolic profile of NIE115 cells is affected by adrenergic pharmacological intervention. Methods: NIE115 cultures (n = 15) were dual- or triple-labelled with antibodies directed against AR1 as well as specific neuronal markers (neurofilament, TRPV1, TUJ1, CGRP). Cells were then exposed to an AR1 agonist (phenylephrine, n = 18), an antagonist (prazosin, n = 12) or a combination of both (n= 12) and the biochemical effects studied using gas chromatography-mass spectrometry-based metabolomics. Results: Immunohistochemistry confirmed the presence of AR1 on the NIE115 cells. Treatment of the cells with phenylephrine led to changes in both carbon and nitrogen metabolism consistent with stimulation of AR1. It was expected that prazosin would block the metabolic effects of phenylephrine, but a different set of changes to carbon and nitrogen metabolism were observed. This provides further evidence to the observations that prazosin may in fact be acting as an inverse agonist. Conclusion: These data indicate that metabolomics is a powerful technology in the study of receptor signaling, and have provided us with a new tool to investigate CRPS.

POS-MON-192

THREE CA2+ CHANNEL INHIBITORS IN COMBINATION REDUCE THE MYELIN DECOMPACTION AND FUNCTIONAL LOSS OF SECONDARY DEGENERATION IN VIVO, FOLLOWING WHITE MATTER INJURY

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Purpose: Secondary degeneration of nerve tissue adjacent to a traumatic injury results in further loss of neurons, glia and function, via mechanisms thought to be initiated by excess Ca2+ influx. Methods: Using an in vivo model of secondary degeneration involving partial transection of the optic nerve of adult rat, we assessed efficacy of every possible combination of three Ca2+ channel inhibitors, to inhibit excess Ca2+ entry into neurons and glia by multiple routes. We used lomerizine (delivered orally, daily), YM872 and/or oxATP (both delivered by osmotic mini-pump for the first 2 weeks after injury) to block voltage gated Ca2+ channels, Ca2+ permeable AMPA receptors and purinergic P2X2 receptors respectively, assessing outcomes at 3 months. Results: Most of the treatment combinations were effective to varying degrees at preserving immunoreactivity of myelin basic protein, GFAP**astrocytes, retinal ganglion cell axons and somata. However, electron microscopy analysis of myelin ultrastructure revealed that only treatments including lomerizine reduced the proportion of axons with decompacted myelin and increased the proportion of normally myelinated axons to levels not different from normal (p<0.05). Importantly, only treatment with all three agents together reduced the density of axons with partially decompacted myelin and preserved astrocytes. Similarly, treatment with all three inhibitors together was required to completely preserve visual function, as assessed using the optokinetic nystagmus reflex (p<0.05). Conclusion: Lomerizine, YM872 and oxATP delivered in combination may serve as a therapeutic intervention for treatment of secondary degeneration following neurotrauma.
POSTERS

POs-Mon-193

EARLY MOLECULAR CHANGES IN THE LYSOSOMAL STORAGE DISEASE FUCOSIDOSIS

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Fucosidosis, caused by a deficiency of α-L-fucosidase due to mutations in the FUCA1 gene is a severe neurovisceral lysosomal storage disease inherited in chickens and English Springer spaniels. Canine fucosidosis is the only available animal model of this disease and characterisation of the cellular pathology has revealed that lysosomal vacuolation of neurons and glia, neuronal apoptosis, axonal dystrophy, neuroinflammation and myelin loss occur as early as 8 weeks of age, prior to clinical signs at approximately 6 to 8 months. Purpose: To investigate the molecular pathophysiology in affected dogs less than 6 months old. Methods: Studies were performed on neural tissue from preclinical fucosidosis-affected dogs (n=7) and age-matched unaffected controls (n=3). These included gene expression profiling of the frontal cortex, characterisation of oligodendrocyte and myelin abnormalities in the central nervous system and examination of cytokine expression in the cerebrospinal fluid (CSF). Results: Cytokine (CCR1, CCR5) and cathepsin (CTSC, CTSD, CTSS) genes contributing to neuroinflammation and apoptotic processes were upregulated in preclinical fucosidosis frontal cortex and myelin genes (CNP, MAG, MAL, OPALIN) were downregulated (P<0.05). In follow up examinations, oligodendrocytes demonstrated vacuolation and there was a significant (P<0.001) 67% reduction in oligodendrocyte populations in the corpus callosum, consistent with hypomyelination. MCP-1/CCL2 and KC/CXCL1 were chemokines that showed significant (P<0.001) and rapid elevations in CSF over a 2 month period from 8 to 16 weeks in affected dogs compared to controls. Conclusions: These studies have demonstrated that the molecular pathogenesis of fucosidosis begins well before the onset of clinical signs and further emphasise the need for early treatment intervention. Chemokines MCP1/CCL2 and KC/CXCL1 are also candidates for biomarker development.

POs-Mon-194

THE ROLE OF MICROGLIA IN GLOBLASTOMA MULTIFORME PATHOGENESIS: INFLUENCE OF CYCLOPHILIN A AND CD147 MEDIATED SIGNALLING

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Purpose: Glioblastoma multiforme (GBM) is a common, invariably fatal and aggressive astrocytoma typified by progressive and diffuse infiltration of normal brain tissue. There is an urgent need to elucidate the factors driving tumour progression for treatment development. The severity of GBM has been correlated with both elevated CD147 receptor expression and its receptor binding protein cyclophilin A (CypA). In this study, we aimed to determine if: i) microglia (brain resident macrophages) can secrete CypA, and ii) extracellular CypA can influence GBM cell signalling and migration. Methods: BV2 microglia cell cultures were treated with LPS (1 μM) or the oxidative stress inducer LY83583 (1 μg/ml), and at specific times culture media collected and subjected to western analysis for CypA protein. BV2 microglia were also treated with recombinant CypA protein (1-1000 nM) in a transwell boyled chamber to assess cell migration after 24h. U251 glioma cells were treated with CypA protein (100 nM) and cell lysates subjected to western analysis for Akt phosphorylation. Results: i) BV2 microglia are capable of secreting CypA in response to LPS and LY83583; ii) CypA protein (100 nM) significantly increased BV2 cell migration (p<0.003; n=3); iii) CypA protein (10 nM and 100 nM) reduced basal microglial cell death after 72h (p<0.06; n=3); and iv) CypA protein induced Akt phosphorylation in U251 GBM cells. Conclusion: These findings support our hypothesis that CypA secretion by microglia may play a role in CD147 receptor mediated GBM tumour growth.

POs-Mon-195

HYPOXIC POSTCONDITIONING HAS LONG-TERM NEUROPROTECTIVE ACTIONS FOLLOWING HYPOXIC-ISCHEMIC BRAIN INJURY IN NEONATAL RATS

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Purpose: Recent studies have demonstrated the protective actions of hypoxic postconditioning following focal ischaemic stroke in the mouse and ischaemia in rat cardiomyocytes. The postconditioning phenomenon has evolved from the well described technique of hypoxic preconditioning; in which tolerance to injury is due to the hypoxia-inducible transcription factor (HIF-1), which results in the de novo induction of a number of neuroprotective factors. The long term neuroprotective effects of hypoxic postconditioning following focal ischemic stroke in the neonatal rat have not been explored. Purpose: To investigate the long term neuroprotective effects of hypoxic postconditioning following hypoxic-ischemic (HI) brain injury in the neonatal rat. Methods: On postnatal day 7, Sprague-Dawley rat pups underwent unilateral common carotid artery ligation in combination with 3 hrs at 8% oxygen. Hypoxic postconditioning consisted of 1 hr of 8% oxygen starting 24 hrs post-injury, once a day for 5 days. At 5 weeks post HI, behavioural testing was performed and brains removed for histological analysis. Results: Hypoxic postconditioning (n=12) was shown to improve forelimb grasping strength by 128% (p<0.005, t-test) after HI and reduce the number of foot faults/min detected in a grid walking task by 41% (p=0.05, t-test), compared to HI alone (n=12). In addition, novel object recognition improved after HI in postconditioned animals, with a 121% increase in discrimination index, compared to HI alone (p<0.005, t-test). Furthermore, hypoxic postconditioning was shown to significantly reduce the volume of brain injury following HI by 119% (p<0.0001, t-test). Conclusion: This new finding of the long-term histological neuroprotection accompanied by functional improvements by hypoxic postconditioning further demonstrates the clinical potential of mild hypoxia for the treatment of HI brain injury.

POs-Mon-196

LONGITUDINAL FUNCTIONAL AND CONNECTIVITY CHANGES DURING WORKING MEMORY PERFORMANCE IN HUNTINGTON’S DISEASE: THE IMAGE-HD STUDY

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Purpose: Functional changes in the brain have been shown in both premanifest (pre-HD) and symptomatic Huntington’s disease (sympt-HD) individuals, during both cognitive and motor task performance. The IMAGE-HD study aims to improve understanding of functional brain reorganization during pre-HD, via functional magnetic resonance imaging (fMRI). This investigation focuses on longitudinal working memory using a modified version of the n-BACK paradigm. Methods: Participants were recruited as part of IMAGE-HD and were assessed at baseline and 18 months, n=70 of whom were included in this analyses (n=19 sympt-HD, n=28 pre-HD and n=23 controls). Results: Behavioural results revealed sympt-HD showed a significant decline in performance over time (0-BACK only), and longer response times (0-BACK and 1-BACK) compared with controls. Where controls and sympt-HD showed decreased BOLD activation at 18 months in frontal and parietal regions (parietal only for sympt-HD), pre-HD showed significantly increased BOLD activation across a number of cortical and subcortical regions. Pair-wise correlation based functional connectivity analysis was used to evaluate synchronicity in neuronal activity between various cortical and subcortical regions that showed robust activation during 1-BACK performance (i.e., prefrontal, posterior, parietal, caudate, and cingulate areas). We found a significant longitudinal reduction in connectivity only for the pre-HD group, specifically between pre-frontal and parietal regions with anterior cingulate. Conclusions: These findings demonstrate that functional and connectivity changes occur well before disease onset. The increased functional activation, taken together with decreased functional connectivity, may offer new and important insights on brain compensation during premanifest stages of the disease.
POS-MON-197

GIVING BIRTH TO SEIZURE SUSCEPTIBILITY: METABOLIC DISCREPANCIES IN SEIZURE-PRONE VERSUS SEIZURE-RESISTANT RATS

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Purpose: Epilepsy and autism spectrum disorder (ASD) are highly comorbid conditions. Clinical evidence has shown that hallmark symptoms of both disorders often respond to dietary manipulation, suggesting metabolic factors may be involved in aspects of disease presentation. Accordingly, fatty acid deficiencies have been confirmed in our seizure-prone, ASD-like (FAST) versus seizure-resistant (SLOW) rat strains, despite maintenance on an identical diet. This study further examined metabolic discrepancies between these strains in their natural state and then investigated whether they continue throughout pregnancy, and thereby have the potential to impact fetal development. Methods: Male (12/strain) and virgin (8/strain) versus embryonic day 20 pregnant (10/strain) female FAST and SLOW rats underwent metabolic cage measurements, an intraperitoneal glucose tolerance test (IPGTT) and an insulin challenge. Pancreas islet morphology and β-cell mass were also compared. Results: Despite higher food and water consumption in FAST rats, non-fasted resting glucose levels were lower and glucose clearance was faster in response to a glucose load. FAST rats also showed significantly higher insulin secretion in response to a glucose load. Pregnancy amplified the metabolic differences observed. Finally, β-cell mass was found to be near double in FAST versus SLOW rats. Conclusions: Given the effectiveness of high fat diets in each of these conditions, the observed differences in metabolic profiles may be linked to the relative seizure disposition and contrasting behavioral patterns characteristic of FAST and SLOW rats. Exaggeration of those differences during pregnancy may serve to program fetal metabolism in a way that supports continuation of a seizure-prone, ASD-like state.

POS-MON-198

EXPRESSION OF THE MERTK RECEPTOR IS ALTERED IN MULTIPLE SCLEROSIS

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PURPOSE: Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS). We have previously shown the MERTK receptor to be genetically associated with the risk of developing MS. We therefore investigated whether expression of MERTK, including a splice variant thought to produce soluble MERTK (sMERTK), was altered in MS. METHODS: We used an Affymetrix microarray to examine the expression of the MERTK in T-cells from first demyelinating event (FDE) patients and matched controls (n=11). We then used qPCR to assess the expression of full-length MERTK and splice variant MERTK (vMERTK) in a separate cohort of FDE patients (n=7), as well as in lesions from MS patients (N=10). RESULTS: Using either microarray or qPCR, we found no significant difference in the expression of full-length MERTK (p>0.05) in T-cells derived from FDE patients compared with controls. However, we observed decreased expression of vMERTK in T-cells derived from FDE patients (p=0.04). In contrast, we observed a significant increase in the expression of full-length MERTK in lesions from MS patients (p=0.04). CONCLUSION: We have shown that MERTK expression is altered during the clinical course of MS, in both the periphery and CNS, potentially linking levels of expression with disease pathology. We observed a reduction in the expression of vMERTK in MS patients, potentially indicating a difference in the amount of circulating sMERTK. To further these investigations, we are currently using ELISA to examine the level of sMERTK in plasma in MS patients compared with controls.

POS-MON-199

COMPLEMENT SIGNALLING IN NEUROINFILTRATION AND EXPERIMENTAL MODELS OF PARKINSON’S DISEASE

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Background: Parkinson’s disease (PD) is the second most common neurological condition in Australia affecting one in 350 people. A sustained neuroinflammatory response accompanies dopaminergic degeneration in PD and has been implicated in exacerbating disease pathology and progression. The complement system is an important component of the innate immune system and is a key mediator of inflammation. Increased complement immunoreactivity has been observed in post-mortem PD tissue and thereby have the potential to impact fetal development. Methods: Male (12/strain) and virgin (8/strain) versus embryonic day 20 pregnant (10/strain) female FAST and SLOW rats underwent metabolic cage measurements, an intraperitoneal glucose tolerance test (IPGTT) and an insulin challenge. Pancreas islet morphology and β-cell mass were also compared. Results: Despite higher food and water consumption in FAST rats, non-fasted resting glucose levels were lower and glucose clearance was faster in response to a glucose load. FAST rats also showed significantly higher insulin secretion in response to a glucose load. Pregnancy amplified the metabolic differences observed. Finally, β-cell mass was found to be near double in FAST versus SLOW rats. Conclusions: Given the effectiveness of high fat diets in each of these conditions, the observed differences in metabolic profiles may be linked to the relative seizure disposition and contrasting behavioral patterns characteristic of FAST and SLOW rats. Exaggeration of those differences during pregnancy may serve to program fetal metabolism in a way that supports continuation of a seizure-prone, ASD-like state.

POS-MON-200

NF-κB PATHWAY IN MULTIPLE SYSTEM ATROPHY AND PARKINSON’S DISEASE

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Purpose: Increased NF-κB expression occurs in multiple system atrophy (MSA) and Parkinson’s disease (PD). This study aims to determine whether degenerative α-synuclein aggregates found in these diseases associate with increased NF-κB and a decrease in its upstream inhibitor IκB-α. Methods: Following institutional approvals, brain samples from 7 MSA, 10 PD and 15 matched controls were received from the Australian Brain Bank Network. TBS-soluble and SDS-soluble proteins were extracted from frozen putamen and white matter under the motor cortex and NF-κB, α-synuclein and IκB-α measured using semiquantitative western immunoblotting. Routine double immunofluorescence for NF-κB, α-synuclein and IκB-α was performed on formalin-fixed paraffin-embedded sections from the putamen, midbrain and pontine white matter and assessed using confocal imaging. Results: NF-κB protein was significantly increased in both fractions in MSA compared to controls, with a trend for an increase in the more soluble TBS fraction in PD cases. There was more insoluble NF-κB protein in both controls and MSA (note there is significantly more insoluble NF-κB in MSA), while in PD there was more soluble NF-κB protein. There was a decrease in α-synuclein solubility in MSA but not PD, whereas both MSA and PD had a decrease in IκB-α solubility. Colocalization experiments revealed some overlap between α-synuclein-immunoreactive inclusions and IκB-α-immunoreactivity in both diseases. Conclusions: This data show that both diseases have an increase in NF-κB solubility associated with a decrease in IκB-α solubility with some colocalization of IκB-α in α-synuclein-immunoreactive inclusions. The data suggest an interaction between abnormal α-synuclein and IκB-α, indicating that enhancing IκB-α solubility may be a novel therapeutic target for MSA and PD.
POS-MON-201

SYNTHESIS AND RECEPTORS OF SPHINGOSINE 1-PHOSPHATE IN PERIPHERAL NOCICEPTION

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Purpose: The bioactive lipid sphingosine 1-phosphate (S1P) is involved in a multitude of important signalling cascades including nociceptive pathways. In recent experiments we showed that both S1P-generating enzymes, Sphk1 and 2, and three out of five S1P receptors (S1PR1-3) are expressed in murine dorsal root ganglia (DRG). Interaction of S1P with the S1PR1 sensitizes nociceptive neurons and reduces the nociceptive behaviour in response to inflammatory pain. Here we investigated the role of the S1PR generating enzymes and the S1PR3 in peripheral nociception. Methods & Results: We analysed the behaviour of mice deficient in Sphk1, Sphk2 and S1PR3 and C57/B6 wild-type (wt) mice in response to CPA-induced inflammation. No differences in thermal hyperalgesia or mechanical sensitivity were found between Sphk1−/− and wt-mice (n = 10). In contrast, the absence of Sphk2 modulated the behavioural response to inflammation. Sphk2−/− deficient mice showed increased sensitivity to heat at baseline levels (n = 10) and an inflammation-dependent increase in mechanical sensitivity in the non-inflamed hindpaw (n = 6). These mice also showed differences in the neurochemical profile of DRG neurons in response to nerve damage and differences in the pattern of epigenetic markers in the spinal cord dorsal horn (n = 4-6). Mice with deficiency in the S1PR3 showed reduced spontaneous pain behaviour, isolated DRG neurons a reduction of S1P-induced depolarisation probably via reduced activity of excitatory chloride channels (n = 8-10). Conclusion: The data show that the Sphk-S1P signalling pathways are involved at different levels and this system offers potential targets for the management of inflammatory pain.

POS-MON-202

TYROSINE HYDROXYLASE PHOSPHORYLATION IN NUCLEUS ACCUMBENS FOLLOWING NERVE INJURY

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Purpose: Sciatic nerve constriction evokes pain in all injured rats, yet triggers changes in social behaviour in only a subset. We have reported in injured rats with behavioral change (disability), a decrease in the expression of tyrosine hydroxylase immunoreactivity (TH-ir) in the nucleus accumbens (NAcc). To determine whether this reflects decreased dopamine availability in the NAcc, we sought to quantify the degree of TH-phosphorylation in the NAcc of rats with and without disability after injury. Methods: Phosphorylation of TH serine-19 and TH-ir was evaluated using standard immunohistochemical techniques in serial coronal sections of the NAcc. Brains of rats with (N=6) and without (N=4) disability following nerve injury, defined by reductions in dominance in a resident-intruder, social interaction test, were compared. Staining intensities in the core and shell regions of NAcc were quantified, using image analysis software from digital images. Results: At each of the rostrocaudal levels of the NAcc analysed, bilaterally, and within both core and shell regions, there was a significant correlation (p<0.05), between the intensity of TH-ir and the expression of dominance behaviour. Only, at the most rostral pole of the NAcc there was a significant correlation between the intensity of TH-p-ser19-ir with dominance behaviours, which was again on each side of the brain, and in core and shell regions (p<0.05). Injured rats with disability had the highest ratios of TH-p-ser19-TH. Conclusion: These data raise the possibility that rats with disabilities may have lower availability of dopamine in the NAcc, which may contribute to the disrupted social behaviours observed in this subset of nerve injured animals.

POS-MON-203

AAV VECTOR-MEDIATED ELEVATION OF N-ACETYL ASPARTATE IN THE BRAIN OF WILDTYPE MICE CAUSES SPECIFIC ASPECTS OF THE CANAVAN DISEASE PATHOLOGY

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Background: Canavan Disease (CD) is an autosomal recessive neurodegenerative disease and is characterized by CNS demyelination, psychomotor retardation, and premature death. CD is caused by null mutations of the gene encoding aspartoacylase (ASPA) which normally breaks down its substrate N-acetyl aspartate (NAA) into acetate and aspartate specifically in oligodendrocytes. NAA is produced in neurons by the N-acetyltransferase 8-like (NAT8L) enzyme and is highly enriched in the CD brain. The aspects of CD pathology that are attributable to ASPA-deficiency and those due to increased NAA have not been distinguished. Purpose: The aim of this study was to dissect the effects of chronically elevated NAA from the consequences of ASPA-deficiency. Methods: The open reading frame encoding the FLAG epitope tag fused to NAT8L was cloned into an adeno-associated virus (AAV) expression construct. For in vivo studies we delivered neurotropic AAV vectors carrying the NAT8L or the GFP CDNA bilaterally to the hippocampus of adult wild-type mice. The brains were then subjected to histological and metabolic analysis and compared with ASPA-deficient mutants. TH NMR spectroscopy was used to quantify the concentration of NAA and other metabolites in cell lines and ex vivo. Results: Functional integrity of the construction was confirmed by transient transfection of the AAV-nat8l plasmid into HEK cells and detection of ectopic NAT8L protein by immunoblotting and immunocytochemistry. NAT8L-expressing cells showed high levels of NAA while extracts of controls did not. At one week post vector administration, we could detect robust transgene expression, elevated NAA, and vacuolization specifically in the pyramidal cell layer. Three weeks following stereotaxic delivery of AAV-nat8l vectors to the hippocampus, the pyramidal cells had degenerated, indicating selective death of transduced neurons. Conclusion: Short-term overexpression of nat8l leads to excess NAA and region-specific vacuolization in mouse hippocampal neurons in ASPA-null mice but rapid neuronal death is specific to nat8l-overexpressers.

POS-MON-204

LOCALIZATION OF THE SODIUM CHANNEL β1 SUBUNIT AND ITS REDISTRIBUTION IN A GENETIC EPILEPSY

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Purpose: Our earlier work showed that the β1 subunit of voltage-gated sodium channels (Naβ1) can modulate the excitability of α-subunits at the axon initial segment (AIS). A C121W mutation in the SCN1B gene coding for Naβ1, identified in human epilepsy, was introduced into a mouse model and replicated the human genetic and neuropathological features. Here, we examined expression of Naβ1 at the AIS of subicular neurons in wild-type mice and mice with C121W-based epilepsy. Methods: Immunohistochemistry was carried out on brain tissue from wild-type (n=3), heterozygous (CW; n=3) and homozygous WW mice in either pyramidal or GABAergic subicular neurons. Immunofluorescence of Naβ1 at the AIS of subicular neurons in wild-type mice and mice with C121W-based epilepsy. Results: Immunohistochemistry was used to capture high-resolution images of subicular neurons. At each of the rostrocaudal levels of the subicular neurons analysed, we found that the density of Naβ1 immunoreactivity (IR) was significantly reduced in WW subicular neurons compared to normal controls when quantified at the AIS of both pyramidal and GABAergic neurons in the subiculum of wild-type and heterozygous CW mice. Particularly strong expression of Naβ1 was seen in the distal AIS of GABAergic interneurons (including those positive for parvalbumin), with proximal expression of Naβ1 at the AIS of parvalbumin-negative interneurons. Immunohistochemical staining was used to examine high-resolution images of subicular neurons. Results: Naβ1 subunit was observed at the AIS of both pyramidal and GABAergic neurons in the subiculum of wild-type and heterozygous CW mice. Particularly strong expression of Naβ1 was seen in the distal AIS of GABAergic interneurons (including those positive for parvalbumin), with proximal expression of Naβ1 at the AIS of parvalbumin-negative interneurons. Conclusion: This study is the first to identify Naβ1 at the AIS of GABAergic interneurons. This result implicates Naβ1 expressed in inhibitory neurons in the pathophysiological mechanism leading to epilepsy in patients and animals with the C121W mutation.
POS-MON-205

DEVELOPMENT OF CEREBELLAR PATHOLOGY IN THE CANINE MODEL OF MUCOPOLYSACCHARIDOSIS II A

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There is currently little information relating to the timing of cerebellar pathology and its association with the onset of clinical signs (ataxia and hypometria) in the mucopolysaccharidosis II A (MPS II A) Hushaway dog model. Purpose: to characterise the timecourse of accumulation of primary (heparan sulphate-derived oligosaccharides) and secondary-stored (GM3) substrates in the dog brain, and relate it to the onset of clinical signs. To elucidate underlying mechanisms that potentially lead to Purkinje cell (PKC) death, neuroinflammation, neurodegeneration and calcium homeostasis were also examined. Methods: unaffected heterozygous (n=8) and MPS II A (n=10) dogs ranging in age from 2.2-67.5 months and 2.2-46.9 months, respectively, were available for this study. Fixed paraffin-embedded or frozen cerebellar tissues were examined using histological methods or tandem mass spectrometry. Results: our study revealed that PKC were present up to and including 30.9 months of age but were lost precipitously thereafter, coinciding with the onset of clinical signs. Primary and secondary substrate accumulation and inflammation were detected in the cerebellum early in disease (2.2 months) and decreases in the calcium-binding protein, calbindin-D28K, in PKC, suggested of altered calcium homeostasis, occurred well before the loss of PKC. Calbindin-D28K and GAD65/67-positive axonal spheroids were also observed in the deep cerebellar nuclei and white matter tracts, however, no degenerating neurons or apoptotic cells were identified. Conclusion: the early presence of stored substrates combined with chronic inflammation, axonal spheroids and decreases in calbindin-D28K in the cerebellum of the MPS II A dog suggests that aberrant cellular function and neuronal-glial interactions may contribute to PKC death.

PO-MON-206

PH MODULATION OF SEIZURE ACTIVITY

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Purpose: In patients and animal models, increased brain pH elicited by hyperventilation or bicarbonate can induce seizures. In contrast, reducing brain pH by carbogen gas inhalation (5%CO2-95%O2) can prevent seizures. We developed a mouse model based upon a human epilepsy R43Q mutation in the GABA\(_{\gamma}2\) subunit that recapitulates two key patient phenotypes: febrile seizures (FS) and absence seizures. We investigated how pH modulates these seizure types and hippocampal neuronal network excitability in brain slices from R43Q mice. Methods: FS susceptibility: R43Q mice were placed into a 41°C chamber, breathing room-air (n=6) or carbogen gas (n=8) and latency to first seizure measured. EEG recordings: R43Q mice were implanted with EEG electrodes and number of spike-wave discharges (SWDs) recorded breathing room-air or carbogen gas (n=5) or following 15mmol/kg (i.p.) bicarbonate injection (n=2). In vitro electrophysiology: Oscillations induced by tetanic stimulation of the CA1 stratum oriens, were recorded in the stratum pyramidale in slices from R43Q mice (n=8) at pH 6.9, 7.4 and 7.9. The number of spikes was determined. Results: R43Q mouse FS susceptibility was reduced by carbogen gas compared to room-air; latency to seizure onset was increased (p<0.05). Carbogen gas did not reduce the number of SWDs, induced by bicarbonate injection, compared to room-air. However, in R43Q mice (p>0.05) but as might be expected injection of bicarbonate increased the number of absence seizures. In vitro neuronal network excitability, indicated by number of spikes, was reduced at pH 6.9 and increased at pH 7.9 compared to pH 7.4 (p<0.05). Conclusion: In a mouse model of human genetic epilepsy, pH modulation can alter in vivo epileptiform activity and in vivo seizure susceptibility.

POS-MON-207

CENTRAL ADMINISTRATION OF HISTAMINE H1 RECEPTOR AGONIST, FMPH, BLOCKS OLANZAPINE-INDUCED ACTIVATION OF AMPK-CPT1 SIGNALING IN THE DORSAL VAGAL COMPLEX IN RATS

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Olanzapine treatment is associated with severe obesity. The AMP-activated protein kinase (AMPK) in the dorsal vagal complex (DVC) plays an important role in feeding regulation, which can regulate energy balance through modulating acetyl-CoA carboxylase (ACC)-carnitine palmitoyltransferase 1 (CPT1) signaling. Histamine H1 receptor (H1R) antagonism acts as a key contributor for olanzapine-induced obesity. Aim: To investigate the effect of olanzapine on food intake and DVC AMPK-CPT1 signaling, and whether these effects could be blocked by central H1R activation. Methods: Rats were treated with olanzapine (1 mg/kg, i.d., orally, n=5-8/group) or vehicle for 4 days. On day 5th, different doses (200nM or 100nM) of 2-(3-trifluoromethylphenyl)imidazo[1,2-a]pyridine (FMPH, H1R agonist), or saline was centrally injected before olanzapine or vehicle was given. Cumulative food intake was measured. After 3 days drug wash out period, the treatment was repeated and rats were sacrificed, and DVC tissues were collected for the detection of phosphorylated AMPK, phosphor-ACC levels and CPT1 activity. Results: Olanzapine-induced hyperphagia after 4 days treatment was significantly reduced by FMPH in a dose-dependent manner up to 16hrs (p<0.05). The DVC phosphor-AMPK and phosphor-ACC levels as well as CPT1 activity was significantly increased by olanzapine (p<0.05). The stimulatory effect of olanzapine on phosphor-AMPK tended to be blocked by FMPH 200nM (p=0.06). The up-regulated CPT1 activity induced by olanzapine was significantly inhibited by FMPH 200nM (p<0.05). Conclusion: These findings indicated that activation of AMPK-CPT1 signaling induced by H1R antagonism may play an important role in olanzapine-induced hyperphagia.

POS-MON-208

BRAIN GENE EXPRESSION CHANGES RELATED TO NEURODEGENERATION WITH BRAIN IRON ACCUMULATION IN A MOUSE MODEL OF HAEMOCROMATOSIS

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Purpose: To obtain clues to mechanisms by which iron overload affects the brain, we investigated changes in the brain transcriptome of a mouse model of the iron overload disorder haemochromatosis. Methods: Male wildtype and Hfe\(^{-/-}\)x\(\gamma\)2AKR mice were sacrificed at 12 weeks after 3 weeks on high iron diet (2% carbonyl iron). Brain iron was assessed by a range of techniques (inductively coupled-atomic emission spectroscopy, Perl’s stain, non-heme iron, ferritin immunoblotting). Brain transcripts were investigated by microarray. Following normalisation using Average or Cubic Spline and identification of differentially expressed probes, pathway analysis was performed using the single gene enrichment tool DAVID. Results: All brain iron measures were significantly higher in Hfe\(^{-/-}\)x\(\gamma\)2AKR mice compared to age- and gender-matched controls (\(>1.7\)-fold increase, \(p<0.05\)). Microarray analysis showed 760 probes differentially expressed in mutant mice compared to wildtype (\(p<0.05\)). There was significant over-representation (Benjamini corrected \(p=0.0073\)) of genes important in MAPK signalling, including Pla2g6. This gene is causatively linked to the severe family of neurogenetic diseases Neurodegeneration with Brain Iron Accumulation (NBIA) and showed significantly decreased expression (\(p=0.01\)), as did three other NBIA-linked genes, F2h (\(p=0.00\)), C1orf12 (\(p=0.02\)) and Atl3a2 (\(p=0.04\)). Real-time RT-PCR confirmed these findings. Conclusion: Both changes in the common iron disorder haemochromatosis are likely to involve similar molecular systems to those genetically linked to severe NBIA diseases.
POS-MON-209

ACETYLYATION OF HISTONE 3 AT LYSINE 18 IN THE DORSAL HORN OF THE SPINAL CORD IN RESPONSE TO PERIPHERAL INFLAMMATION – ABSENCE OF SPHINGOSINE KINASE 2

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Purpose & Methods: Differences in pain susceptibility and the development of pathological pain states might be connected by variations in the epigenetic regulation of nociceptive processing. We compared the patterns of histone H3 proteins that are acetylated on lysine 18 in spinal cord 3-7 days after induction of CFA-induced peripheral inflammation in C57Bl6 wild-type and sphingosine kinase 2 deficient (Sphk2/-/-) mice. H3K18ac is a marker of active chromatin. GFAP, Iba-1 and NeuN were used to label astrocytes, microglia and neurons; DAPI was used as a nuclear stain. Positive labelling was analysed using Image J. Sensitivity to mechanical stimuli was tested with von Frey hairs. Results: Compared with C57Bl6 wild-type (wt), Sphk2/-/- mice showed a similar decrease in mechanical threshold on the inflamed side but surprisingly their threshold also decreased on the un-inflamed side (n = 6). Peripheral inflammation as indicated by the weight drop was similar between wt and Sphk2/-/- mice. Multiple labelling immunohistochemistry showed the highest H3K18ac immunoreactivity (IR) in the nuclei of neurons, astrocytes and microglia in the dorsal horn compared to the remaining spinal cord in wt and Sphk2/-/- mice. The overall number and the number of hyperacetylated H3K18ac-IR nuclei was higher in the contralateral spinal cord of Sphk2-/- compared to wt (n = 3–5). Sphk2-deficient mice showed an increased mechanical sensitivity in ipsi- and contralateral hindpaws in response to inflammation which was accompanied by higher H3K18 acetylation levels contralateral. Conclusion: The results suggest a Sphk2-mediated modulation of H3 acetylation levels that modulate spinal cord pain processing.

POS-MON-211

TDP-43 AND FUS ACCUMULATION INTO STRESS GRANULES VIA MECHANISMS OF CELL STRESS

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Purpose: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease which can be caused by mutations in FUS or TDP-43; these proteins are found in cytosolic accumulations in neurons in ALS. Stress granules, or stress granule related mechanisms are believed to be precursors to the neuronal inclusions seen in ALS. FUS and TDP-43 levels in the cytosol increase in response to cell stress, and can accumulate in stress granules. Stress granules are diverse, therefore it is essential to characterise different stress inducers and determine a relationship with FUS and TDP-43 accumulation into specific stress granule populations. Methods: Primary murine cortical neurons and SH-SY5Y cells were grown in culture. Cells were treated with various inducers of cell stress, including sodium arsenite inducing ATP depletion, and hydrogen peroxide inducing oxidative stress, subsequently cells were either fixed for immunofluorescence, or used in assays measuring cell stress, (i.e., oxyblot analysis, ATP analysis). Results: Inducers of cell stress significantly (n≥3, P≤0.05) enhanced FUS and TDP-43 movement to the cytosol, with subsequent accumulation into stress granules in both primary cortical neurons and SH-SY5Y cells. This pattern of accumulation varies depending on the type of stress, the cell type and effects of the stress on metabolic and oxidative parameters. Conclusion: In models of cell stress, FUS and TDP-43 levels increase in the cytosol, leading to accumulation of FUS and TDP-43 into stress granules. These stress granules vary dependent on the type of cell stress induced. Knowing the precursor pathways involved in FUS or TDP-43 accumulation could provide insight into the mechanisms of disease pathology.

POS-MON-210

EFFECT OF 670NM PHOTOBIMODULATION ON APOPTOSIS, AXONAL SPROUTING AND INFLAMMATORY CELL PHENOTYPES FOLLOWING HEMI-CONTUSION SPINAL CORD INJURY

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We tested the effect of photobiomodulation (PBM) using 670nm light on cell death, axonal sprouting, macrophage polarisation (M1/M2 ratio) and functional recovery following spinal cord contusion injury. 8 week old male Wistar rats received weight-drop (10g, 25mm) hemi-contusion spinal cord injuries at T10 and divided into sham-treated control (n=15) and 670nm PBM treated groups (n=15) using a LED array. Treatments of 30 min commenced 15 min after the injury and then every 24 h. Spinal cords were collected 1 d (n=6), 3 d (n=6) and 7 d (n=6) after injury for TUNEL to measure apoptosis and immunohistochemistry to label axonal sprouting and macrophage sub-populations. A modified Basso, Beattie, and Bresnahan scale was used to evaluate locomotor recovery. The PBM treated group showed significantly reduced TUNEL density at 3 d (P < 0.01), but not at 1 d or 7 d post-injury, and increased Growth Associated Protein 43 (GAP43) expression at 1 d (P< 0.05) and 7 d (P < 0.05). The PBM treated group showed less CD80 (M1 marker) expression and more Arginase 1 (M2 marker) expression as a percentage of total ED1-positive monocytes than the sham-treated control group in the dorsal white matter ipsilateral to the injury. Locomotor recovery ipsi- and contralateral to the injury were significantly improved by 670nm PBM (P < 0.01). 670nm PBM reduces cell death, promotes axonal sprouting and shifts macrophage polarisation in favour of regulatory (M2) and away from inflammatory (M1) macrophage populations, and together these effects may contribute to improved functional outcomes.

POS-MON-212

REGULATION OF A-SYNUCLEIN IN HUMAN ALCOHOLIC BRAIN

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Purpose: Chronic alcohol misuse results in alterations in gene expression in brain regions susceptible to the neurotoxic effects of alcohol. One such gene, α-synuclein, exists in a number of different splice-variants and its expression is influenced by both genetic factors and microRNAs. Here we investigate the influence of these factors on the expression of α-syn in human alcoholic brain and following exposure of cells to ethanol. Methods: Real-time PCR was used to measure α-syn splice-variant mRNA (SNCA140, SNCA112 and SNCA115) levels in a human neuroblastoma cell line (HEK293T). Comparisons were made between chronic and chronic-intermittent treatment with and without a withdrawal period to determine if the α-syn splice variants are differentially expressed in response to ethanol. mRNA expression was also measured in the human prefrontal cortex of 26 controls, 26 alcoholics, 12 alcoholic cirrhotics. Results: The expression of SNCA140 and SNCA112 was significantly lower in the prefrontal cortex of cirrhotic alcoholics (P = 0.030 and P < 0.001) whereas the expression of SNCA115 was significantly higher in cirrhotic alcoholics (P = 0.006). Results show that the expression of SNCA112 and SNCA115 is up-regulated following ethanol treatment (P < 0.001, n = 16; P < 0.001, n = 16) particularly following withdrawal (P < 0.001, n = 8; P < 0.001, n = 8), whereas the expression of SNCA140 is down-regulated (P < 0.001, n = 16). Our recent studies show that both miR-7 and miR-153 were down-regulated following ethanol exposure (P < 0.001, n = 16). Conclusion: It is likely that these miRNAs act as “master switches” and may be responsible for many of the gene expression changes that occur in the brain of chronic alcoholics.
POS-MON-213

VIRTUAL SCREENING IDENTIFIES LUPEOL AS AN ALLOSTERIC INHIBITOR OF PTP1B

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Purpose: PTP1B is significantly increased in the hypothalamus and plays an important role in central leptin resistance of diet induced obesity in rodents. Leptin signaling pathway is activated by the tyrosine phosphorylation of Jak2. PTP1B negatively regulates leptin signaling via dephosphorylating Jak2. Inhibition of PTP1B in the obesity status may enhance leptin sensitivity, decrease adiposity and prevent excessive weight gain. PTP1B is highly similar to TCPTP. However, deletion of TCPTP in mice leads to immune damage. Current PTP1B inhibitors fail to inhibit PTP1B with high selectivity over TCPTP resulting from targeting the catalytic site. Novel drug design is targeting the allosteric site of PTP1B. Lupeol reduces PTP1B activity in vitro and is potentially a good candidate drug. However the binding site remains unclear. This study presents a combination of docking and molecular dynamics simulations to predict and characterize the binding mode of lupeol to PTP1B.

Methods: Molecular docking and molecular dynamics are applied to predict the binding site and simulate the PTP1B-lupeol complex. Various mutagenesis of specific residue in PTP1B (n=3) are used in enzymatic assay to validate the predicted interactions. Results: The preliminary data confirm lupeol binding to the allosteric site which is close to the former reported allosteric site about 20Å away from the catalytic site. The binding propensities of lupeol and lupeol like compounds are reported. The data also reveal the important hydrogen bonding and non-polar interactions. The results, together with experimental data, suggest that lupeol is an allosteric inhibitor. Conclusion: This research demonstrates lupeol as an allosteric PTP1B inhibitor and attracts more attention to render the lupeol structure to produce more potent and selective inhibitors.

POS-MON-214

INCREASED PERIPHERIN IN SYMPATHETIC AXONS INNERVATING PLANTAR METATARSAL ARTERIES IN DIABETIC RATS - EVIDENCE FOR AXONAL REMODELLING?

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Purpose: Impaired sympathetic neurovascular transmission (NVT) may contribute to vascular-related diabetic complications. We previously demonstrated that streptozotocin (STZ)-induced type I diabetic rats without insulin support had impaired NVT in plantar metatarsal arteries (PMA), which supply blood to the hindpaw digits. This impairment of NVT was not observed in STZ-rats receiving a low dose of insulin (1 unit/day) despite their being severely diabetic (blood glucose>20mM). However, in both groups of PMAs, the morphology of perivascular sympathetic axons was modified. Here, in STZ-treated rats receiving a low dose of insulin, we analysed peripherin-immunoreactive perivascular fibres and peripherin expression because this intermediate filament protein is reported to be increased in regenerating axons. We also assessed the effects of reducing blood sugar levels with a high dose of insulin (4 units/day). Methods: PMAs of STZ-treated rats (60mg/kg, i.p.) receiving a low (n=13) or high (n=13) dose of insulin for 12 weeks were compared to tissues from vehicle-treated controls (n=14). Perivascular axons were co-labelled with antibodies to peripherin and to β-tubulin III or neuropetide Y (NPY). Peripherin protein levels (relative to β-actin) were quantified by western blotting. Results: PMAs from diabetic rats receiving a low dose of insulin (blood glucose 26.5±1.0mM) had increased immuno-labelling of peripherin in axons co-labelled with β-tubulin III or NPY. Furthermore, peripherin protein levels were increased (P<0.01) compared to control. Neither of these changes were observed in diabetic animals receiving a high dose of insulin (blood glucose 9.0±1.9mM). Conclusion: Increased peripherin in sympathetic axons is likely due to hyperglycaemia and suggests axon remodelling. This makes PMAs a suitable model to assess the development/prevention of diabetes-induced sympathetic neuropathy.

POS-MON-215

GAMMA FREQUENCY NEURAL OSCILLATIONS AND PREPULSE INHIBITION: A CASE OF SIGNAL-TO-NOISE?

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Purpose: An emerging literature implicates abnormalities in gamma frequency neural oscillations in the symptoms of schizophrenia. Prepulse inhibition (PPI) is a behavioural measure of sensorimotor gating, and is disrupted in schizophrenia patients. Here we studied the relationship between neural oscillations and PPI in PPI, with the hypothesis that increasing gamma power would lead to an increased ‘noise’ in neural circuits and disrupt PPI. Methods: Adult Wistar rats (n=7) were surgically implanted with extradural recording electrodes. Rats were connected to EEG cables, and placed into PPI chambers, facilitating simultaneous EEG and behavioural measurement. Rats received sc injection of either Ketamine (5mg/kg), MK801 (0.08mg/kg), amphetamine (0.5mg/kg), LY379268 (0.3mg/kg) or vehicle (saline) and were subjected to 90 minutes of PPI trials and EEG recording. Outcomes were measured every 5 minutes. Results: Administration of the 3 psychotomimetic compounds (ketamine, MK801, amphetamine) led to an increase in the power of gamma oscillations and a time-matched disruption of PPI. The three drugs had different kinetics: ketamine showed a rapid onset and offset (within 30 min) of action, MK801 a slowly developing but prolonged effect (>90min), whereas amphetamine elicited a delayed response which was less pronounced than the NMDA receptor antagonists. In contrast, the mGlur agonist LY379268 significantly reduced gamma power, but also disrupted PPI suggesting a dichotomy between behaviour and neural oscillations. Conclusion: The contrasting effects of the drugs studied here (all disrupted PPI but had differing effects on gamma power) is suggestive of a complex relationship between neural oscillations and sensorimotor behaviour. Psychotomimetic drugs increase gamma frequency noise which may be causal to the observed disruption of PPI, whereas LY379268 may be dampening the signal itself.

POS-MON-216

SELECT REDUCTIONS IN BDNF AND TRK-B EXPRESSION IN ORBITAL AND MEDIAL PREFRONTAL CORTEX IN RATS WITH DISABILITY FOLLOWING NERVE INJURY

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Purpose: Exposure to uncontrollable stressors decreases BDNF expression in the prefrontal cortex (PFC) of rats. This response is thought to underlie in part, a persistent failure of stress coping strategies, which manifests as depressive-like behaviour. The incidence of depression with neuropathic pain is high. We therefore sought to determine whether nerve-injured rats that displayed disrupted social behaviour also had reduced expression of BDNF and its receptor TrkB in the PFC. Methods: BDNF and TrkB mRNA and BDNF protein expression levels were determined in medial and orbital PFC of rats with (N=7) and without (N=7) diabetes following nerve injury, defined by reductions in dominance in a resident-intruder, social interaction test. RT-qPCR and western blot techniques were used to quantify mRNA and protein levels respectively. Results: In the medial PFC, BDNF mRNA was down-regulated contralateral to the injury (p<0.03) in diabetic rats, furthermore, TrkB mRNA expression was increased in the same region (p<0.03). In the orbital PFC, BDNF mRNA expression was reduced in rats with disability on each side of the brain (p<0.05), whereas TrkB did not differ in the PFC region. Conclusion: In the medial PFC of diabetic rats, the lateralised down-regulation of BDNF coupled with an up-regulation of TrkB suggests a compromised capacity for neuroprotection, further, the down-regulation of BDNF in the orbital PFC also suggests a vulnerability of neurons in this region. Literature reports of the association of reduced BDNF in the PFC with failure to cope with ongoing stressors, suggests that further behavioural analysis of our nerve-injured rats with disrupted social behaviours may reveal other depressive-like behaviours.
**POS-MON-217**

**NON-IGF-1 RECEPTOR DEPENDENT RESCUE OF ADULT MOTONEURONES BY AN ISOFORM OF IGF-1 ISOLATED FROM ACTIVE MUSCLE**

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**Purpose:** We previously found that transfer of the gene for an isoform of insulin-like growth factor-1 (IGF-1) that is expressed by muscle (termed MGF) rescued 80% of adult rat facial motoneurones when 80% would have died. In this study, we tested the possibility that the MGF peptide is also neuroprotective and whether it acts via the IGF-1 receptor. **Methods:** The right facial nerve was avulsed as it emerged from the stylomastoid foramen in 5 groups of 5 anaesthetised adult Sprague-Dawley rats. In one group, 10μl of 1μg/μl MGF in saline was injected into the foramen immediately after avulsion. In another group, the MGF was co-injected with 1μg/μl of an antibody to the rat IGF-1 receptor. In the remaining groups, MGF was replaced with 1μg/μl glial-cell derived neurotrophic factor (GDNF), the liver-type isoform of IGF-1 or saline only. Rats were perfused with fixative 1 month later and numbers of motoneurones were determined stereologically. **Results:** 1 month following avulsion only or avulsion plus saline, 80% of motoneurones were lost ipsilaterally. This loss was reduced to 20%, 50% and 60% by GDNF, MGF and IGF-1 respectively. Co-injection of MGF with an antibody to the IGF-1 receptor did not affect MGF rescue at 1 month. **Conclusion:** Although not as potent as GDNF, MGF peptide rescues avulsed adult motoneurones and appears not to do this via the IGF-1 receptor.

**POS-MON-219**

**POTENTIAL ROLE OF ABCA8 IN OLIGODENDROCYTE**

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Members of ATP-binding cassette subfamily A (ABCA) are characterized by their ability to transport lipids across cellular membranes and regulate lipid homeostasis in the brain. ABCA8 is a little-known member of this subfamily, originally cloned from human brain libraries, with no known function. In an effort to elucidate the role of ABCA8 in the brain we undertook an analysis of its expression in the human brain and found that ABCA8 was differentially expressed in multiple regions with significantly higher expression in oligodendrocyte-enriched white matter regions compared to grey matter cortical regions. Based on these and other previous data we hypothesized that ABCA8 is involved in either the regulation or transport of sphingomyelin in oligodendrocyte; sphingomyelin is a major lipid component of myelin which is produced by oligodendrocyte. ABCA8 was able to significantly stimulate both sphingomyelin synthesize 1 expression and cellular sphingomyelin production in oligodendrocyte. However, it was not able to transport sphingomyelin in oligodendrocyte under our experimental conditions. These new data suggest that ABCA8 functions as a regulator of sphingomyelin production in oligodendrocyte and may play a role in myelin formation and maintenance.

**POS-MON-220**

**IMAGING NEUROINFLAMMATION USING POSITRON EMISSION TOMOGRAPHY AND THE TSPO**

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**Purpose:** To evaluate the TSPO radiotracer 18F-PBR111 in the relapsing remitting experimental autoimmune encephalomyelitis (RR-EAE), animal model of neuroinflammation and non-human primates using positron emission tomography (PET). **Methods:** RR-EAE mice (n>35) were administered with 18F-PBR111 and brain uptake and disease progression were followed by microPET imaging. The extent of TSPO expression and glia activation was assessed with immunohistochemistry, immunofluorescence and RT-PCR. The kinetics, brain biodistribution and specificity of 18F-PBR111 were also assessed in baboons (n=4) at baseline and after pre-blocking with PK11195 (5 mg/kg) using PET-CT. Arterial plasma samples and metabolite analysis provided the metabolite-corrected input function. **Results:** RR-EAE mice showed large and significant overexpression of TSPO in CNS, consistent with clinical episodes. TSPO expression was associated with microglia and macrophages but without obvious astrocyte labelling. In baboons, brain cortex and bone marrow time activity curves displayed specific binding that could be blocked by PK11195. **Conclusion:** 18F-PBR111 clearly imaged brain microglial activation during RR-EAE. TSPO expression was confirmed by immunohistochemical and PCR techniques. 18F-PBR111 displayed good pharmacokinetics and stability and high target to ratio capable of detecting subtle inflammatory process in animal models of disease. These results suggest a significant role for PET TSPO imaging investigations of neuroinflammation and neurodegeneration.

**POS-MON-222**

**EFFECT OF OXCARBAZEPINE AND ITS DERIVATIVES S-(+)-LCARBAZEPINE AND R-(+)-LCARBAZEPINE ON GENERALIZED AND FEBRILE SEIZURES**

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**Purpose:** Oxcarbazepine is a new antiepileptic that does not form ‘epoxide’ metabolites which are thought to be responsible for some of the side effects including exacerbation of generalized seizures commonly observed with an older derivative, carbamazepine. Oxcarbazepine is instead metabolized to monohydroxy derivatives, S-(+)-licarbazepine and R-(+)-licarbazepine in 4:1 ratio. Our goal was to investigate the effects of oxcarbazepine and its two metabolites in rodent models of generalized and febrile seizures. **Methods:** Spike-and-wave discharges (SWDs) and thermal seizure susceptibility were measured in GABAγ2(R43Q) knock-in mouse model of genetic epilepsy. SWDs were measured on electroencephalograms. Thermal seizure susceptibility was determined by measuring the latency to first tonic-clonic seizure of mice subjected to 42oC. **Results:** Animals were treated with 20mg/kg oxcarbazepine, S-(−)-licarbazepine or R-(−)-licarbazepine. All oxcarbazepine, S-(+)-licarbazepine and R-(−)-licarbazepine significantly increased SWD counts (n=10, 11 and 11, P<0.05). Oxcarbazepine and S-(−)-licarbazepine exacerbated thermogenic seizures with a shorter latency to first seizure when compared to vehicle control (n=8, 8 and 33, P<0.05). In contrast, R-(−)-licarbazepine was protective, significantly increasing the time to first seizure (n=15 and 33, P=0.001). **Conclusion:** Oxcarbazepine, S-(−)-licarbazepine and R-(−)-licarbazepine all exacerbate SWDs suggesting that they may be contraindicated in certain forms of generalised epilepsy. It also suggests that seizure exacerbation caused by carbamazepine is unlikely to be due solely to its epoxide metabolites. Oxcarbazepine also exacerbated thermogenic seizures in our model with S(−)-licarbazepine likely to be responsible given that it also exacerbated seizures and accounts for 80% of the metabolites. Interestingly, R-(−)-licarbazepine was protective against thermogenic seizures potentially making it an effective antiepileptic in febrile seizures.
**POS-MON-221**

**SEX DIFFERENCES IN THE REGIONAL EFFECTS OF MATERNAL SEPARATION AND ADOLESCENT CORTICOSTERONE TREATMENT ON EXON-SPECIFIC BDNF MRNA AND SIGNALLING**

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**Purpose:** Post-mortem studies have demonstrated reduced expression of brain-derived neurotrophic factor (BDNF) in schizophrenia. However, the role of developmental stress in these changes is unclear. **Methods:** Wistar rats were exposed to neonatal maternal separation (MS) and/or adolescent corticosterone (CORT) treatment. In adulthood, several regions of the cortex, striatum and hippocampus were analyzed by qPCR for exon-specific BDNF gene expression (n=6/group) or by Western blot for BDNF protein signalling (n=7/group). **Results:** MS and CORT or their combination elicited sex- and region-specific alterations in BDNF mRNA expression or protein levels. For example, in the medial prefrontal cortex (MPFC) MS significantly increased exon II, III, IV, VI and IX in females but not males. In contrast, CORT treatment elicited a significant decrease in exon III, VII and VIII in females in this region. BDNF protein expression was unchanged by MS and CORT. No changes in BDNF transcript levels were found in the nucleus accumbens. In the dorsal hippocampus (DHP) MS caused a male-specific increase in exon I, II, IV, VII, and IX which was accompanied by a decrease in BDNF protein expression. CORT caused a female-specific increase in exon II, III and IV in the DHP, however protein expression levels were unchanged. In the ventral hippocampus (VHP) CORT caused a male-specific increase in exon IV, VII, VIII and IXa. **Conclusion:** These data provide intricate details of the effects of developmental disruptions on BDNF mRNA and protein expression. These findings add to our understanding of molecular mechanisms that may underlie neurodevelopmental illnesses such as schizophrenia.

**POS-MON-222**

**ANTIOXIDANT PROPERTIES OF APOLIPROTEIN-D METHIONINE- AND SELENOMETHIONINE-CONTAINING PEPTIDES**

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Apolipoprotein D (ApoD), a lipocalin abundantly present in the central nervous system, has been recently shown to combat oxidative stress. This protein increases expression during Alzheimer’s disease and other neurodegenerative diseases, indicating that this is one of the body’s natural defences against redox events. The reducing capacity of ApoD is believed to come from Met93, which is exposed within the ligand-binding region of the protein. **Purpose:** We have developed nine peptides based on the three methionines within ApoD to determine the strength of reduction caused by methionine, and whether this is affected by the surrounding amino acids. In addition, selenomethionine mutants have been used in order to establish whether selenium confers a stronger redox capability than sulfur, and whether peptides containing this amino acid can be recycled to perpetuate the antioxidant cycle. **Results:** Initial studies have proved Met49 and SeMet49 to be highly successful reductants, with the SeMet49 peptide demonstrating extremely rapid reduction within the HPLC studies, as well as in experiments using liposomes as a model for membrane lipids. The SeMet93 is capable of bringing the peroxidation of liposomes to baseline level over a 12 hour timecourse.

**POS-MON-223**

**THE EFFECT OF A WESTERN DIET ON THE SYNTHESIS AND METABOLISM OF CHOLESTEROL IN THE BRAIN**

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**Purpose:** Cholesterol is an important molecule in the brain however the effect of diet on cholesterol synthesis and metabolism in the brain has not been characterised. The purpose of this study was to identify levels of cholesterol synthetic precursors, metabolic and oxidation products as well as phytosterols in different brain regions. **Methods:** The effect of a westernised diet on brain levels of these compounds was also investigated. **Method:** A diet study was performed in male C57BL/6 mice, feeding diets high in fat (18% from ghee, 1% cholesterol) and heme (2.5%) over 4, 9 and 20 weeks (n=6 for each diet). Lipids were extracted from collected brain tissue and analysed for 16 compounds using a novel triple quadrupole gas chromatography-mass spectrometry method. **Results:** Significant differences between cortex and cerebellum were detected in 27-hydroxycholesterol and 24-hydroxycholesterol (P<0.0001); lathosterol and 24,25 dihydro lanosterol (P<0.005). Dietary effects were detected with significant increases in the cholesterol oxidation products 7β-hydroxycholesterol (5 fold) and 7-ketocholesterol (2 fold) after 20 weeks of high-fat-heme diet. A significant increase of 27-hydroxycholesterol was also measured in the cerebellum when mice were fed diets containing heme. The phytosterol campesterol accumulated in control brains while high fat containing diets did not show an increase. **Conclusions:** Different mouse brain regions have a specific profile of cholesterol synthetic precursors and metabolites. A westernised diet increases cholesterol oxidation products in the brain as well as the oxysterol 27-hydroxycholesterol. This may indicate increased oxidative stress and/or disruption of the blood brain barrier.

**POS-MON-224**

**AXONAL DEGENERATION IS LIMITED IN THE OPTIC NERVE OF EAE-INDUCED MICE BY AAV2 TRANSDUCTION OF RETINAL GANGLION CELLS (RGCS) WITH A SITE-SPECIFIC PHOSPHO-MUTANT CRMP-2**

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**Purpose:** Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterised by demyelination and axonal degeneration. The molecular mechanisms that underpin axonal degeneration are relatively unexplored in MS. Studies using the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), have shown that the collapse response mediator protein 2 (CRMP-2), which play a significant physiological role in neuronal cell bodies and axons within the CNS, is phosphorylated during the neurodegenerative phase of the inflammatory disease. **Methods:** We investigated the limitation of axonal degeneration by transducing retinal ganglion neurons with a phosphorylation mutant of CRMP-2 utilising an intraocular adeno-associated virus 2 (AAV2) delivery system in EAE-induced mice. The other group of mice injected with AAV2 consisting of the green fluorescent protein reporter only (AAV2-GFP) with EAE was used as a control (n=8 per each group). **Results:** We showed substantial preservation of axons of the optic nerve in the EAE-induced mice injected with the AAV2 carrying the CRMP-2-phospho-mutant compared (~10-fold difference) with those mice injected with AAV2-GFP. The AAV2-GFP transduced axons showed significant degeneration during the peak stage of EAE. **Conclusion:** Our data suggest that phosphorylation of CRMP-2 may be a central mechanism that governs axonal degeneration during inflammatory demyelination of the CNS (as occurs in MS) and inhibition of phosphorylation of CRMP-2 may be of therapeutic potential for the progressive phase of the disease in MS.
POS-MON-225

NPAS4 KNOCKDOWN REDUCES FOREBRAIN NEUROGENESIS

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Purpose: Investigate the role of Npas4 in zebrafish embryos. Npas4 is a brain specific transcription factor, important in neurogenesis and neuroprotection. It specifically regulates genes that determine the number of inhibitory synapses on excitatory neurones. This controls the balance or ratio of inhibitory and excitatory synapses. Studies implicate Npas4 in disorders thought to involve loss of homeostatic between excitation and inhibition. Disorders associated with Npas4 include seizure, autism and schizophrenia. Furthermore, Npas4 is induced by brain injuries or stresses, including seizure, cerebral ischaemia, and cortical spreading depression. Methods: Qualitative spatial and temporal expression of Npas4 was measured by in situ hybridisation and qRT-PCR respectively in zebrafish embryos. Treatment with pentylenetetrazole, a GABA antagonist, was able to test for Npas4 induction. Npas4 Knockdown of Npas4 expression in zebrafish embryos was achieved with anti-sense morpholinos. mRNAs were stained by in situ hybridisation and qRT-PCR respectively. Results: Npas4 appears to function in zebrafish as in mammals. The GABA antagonist PTZ was able to induce Npas4 in embryos. Formation of the embryonic forebrain was disrupted with knockdown of Npas4 expression, and the ventro-dorsal head size was reduced from 131.8 μm to 124.1 μm (p<0.008 n=51), and the lateral surface area reduced from 697 μm² to 583 μm² (p<0.01). Notably dlx1 expression was reduced in these zebrafish morphants. There was no difference in cell division between control and morphant brains, however, neuronal survival was significantly reduced (p<0.03). Conclusion: Zebrfish is a suitable model organism to study the role of Npas4. Expression was observed in embryos despite previous reports in rodent studies. Npas4 is important in formation of zebrafish embryo forebrain.

POS-MON-227

DELETION OF ABCA7 INCREASES CEREBRAL AMYLOID-ß DEPOSITION IN THE J20 MOUSE MODEL OF ALZHEIMER’S DISEASE

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ATP-binding cassette transporter A7 (ABCA7) is expressed in the brain and has been detected in macropores, microglia, and neurons. ABCA7 promotes efflux of lipids from cells to apolipoproteins. ABCA7 can also regulate phagocytosis and modulate processing of amyloid precursor protein (APP) to generate the Alzheimer’s disease (AD) amyloid-ß (Aß) peptide. Genome-wide association studies indicate ABCA7 SNPs confer increased risk for late-onset AD; however, the role that ABCA7 plays in the brain in the AD context is unknown. In the present study we crossed ABCA7 deficient (Aß/-) mice with J20 amyloidogenic mice to address this issue. We show that ABCA7 loss doubled insoluble Aß levels and thioflavine-S positive plaques in the brain. This was not related to changes in APP processing (assessed by analysis of full-length APP and the APP-derived C-terminal fragment). Apolipoprotein E (apoE) regulates cerebral Aß homeostasis and plaque load, however, apoE concentration was not altered by ABCA7 loss. Spatial reference memory was significantly impaired in both J20 and J20Aß/-/- mice compared to wild type mice; however, there were no cognitive differences comparing J20 and J20Aß/-/- mice. There were also no major differences detected in hippocampal or plaque-associated microglial/macroage markers when J20 and J20Aß/-/- mice were compared, whereas the capacity for macrophages derived from Aß/-/- mice to take up oligomeric Aß was reduced by 51% compared to wild type mice. Our studies suggested ABCA7 plays a role in the regulation of Aß homeostasis in the brain and that this may be related to altered phagocytic function.

POS-MON-226

EPIGENETIC MECHANISMS IN GENE TRANSCRIPTION IN AMYLOID-ß PRODUCTION UNDER OXIDATIVE STRESS

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Purpose: Amyloid-ß (Aß) that composes senile plaques plays a causal role in Alzheimer’s disease (AD). In the present study, we investigated the epigenetic mechanisms such as DNA methylation and histone acetylation, involved in the transcription of AD-related genes with Aß production under oxidative stress. Methods: Human neuroblastoma SH-SY5Y cells were treated with hydrogen peroxide (H2O2) and used as the cell model. Sequenom MassARRAY platform was used to perform the quantitative methylation analysis of amyloid-ß precursor protein (APP) and b-site APP-cleaving enzyme 1 (BACE1) genes. Histone H3 and H4 acetylation was measured using EpiQuick Global Histone H3/H4 acetylation assay kit. Electrophoretic gel mobility shift assay was used to test DNA binding activity of nuclear factor-kb (NF-kb) or specific protein-1 (SP-1). Results: The intracellular Aß level was significantly increased in H2O2-treated SH-SY5Y cells (n=6). The expression of APP and BACE1 was up-regulated by demethylation in the gene promoters associated with the reduction of methyltransferases (DNMTs) (n=6). Meanwhile, H2O2-induced the up-regulation of histone acetyltransferases p300/CBP and down-regulation of histone deacetylases (HDACs) (n=6). DNA hypomethylation induced by DNMT inhibitor could activate the DNA binding activity of NF-kb, whereas no significant effect was observed on SP-1 (n=6). Both DNA binding activities of NF-kb and SP1 were activated by histone hyperacetylation induced by HDAC inhibitor (n=6). Conclusion: These findings suggested that oxidative stress resulted in an imbalance between DNA methylation and demethylation as well as histone acetylation and deacetylation associated with the activation of transcription factors, leading to the AD-related gene transcription in the Aß over-production. This could be a potential mechanism for oxidative stress response, which might contribute to the pathogenesis and development of AD.

POS-MON-228

INTRALAMINAR STIMULATION OF THE INFERIOR COLLICULUS FACILITATES FREQUENCY-SPECIFIC ACTIVATION IN THE AUDITORY CORTEX

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Purpose: Auditory midbrain implants (AMI) tested in clinical settings, have provided inadequate frequency discrimination for open set speech perception. AMIs that can take advantage of the tonotopic laminar of the midbrain may be able to better deliver frequency specific perception and lead to enhanced performance. This research examined the characteristic frequency (CF) relationship between the regions of evoked responses in the auditory cortex (AC) to stimulated regions of the auditory midbrain, comparing monopolar and intralaminar bipolar electrical stimulation. Method: Electrical stimulation through microelectrode arrays in the inferior colliculus (IC) was used to elicit AC responses in anaesthetised male Hooded Wistar rats. Results: Changing distance between the stimulation and reference sites in the IC resulted in changes to both threshold and dynamic range with bipolar stimulation and 200 μm spacing providing the highest threshold and narrowest dynamic range. At saturation, bipolar stimulation elicited a significantly higher mean spike count in the AC at CF-aligned areas than at CF-unaligned areas when electrode spacing was 400 μm or less. Bipolar stimulation using electrode spacing of 400 μm or less also elicited a higher rate of elicited activity in the AC in both CF-aligned and CF-unaligned regions than monopolar stimulation. Furthermore, monopolar stimulation of the external cortex of the IC resulted in more localised frequency responses than bipolar stimulation when stimulation and reference sites were 200 μm apart. Conclusion: These findings have implications for the future development of Auditory Midbrain Implants, as a bipolar stimulation strategy may improve the ability of implant users to discriminate between frequencies.
POSTERS

POS-MON-229

THE EFFECT OF SUBTHRESHOLD ELECTRICAL NOISE ON PERCEPTION THRESHOLDS AND DISCHARGE VARIABILITY OF TACTILE AFFERENTS

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Purpose: Previous work has shown that the application of electrical noise to the nerve can improve sensory perception distal to the point of noise application. As the physiological basis of this improvement is unknown, this work aims to establish the underlying mechanism. Method: Unitary recordings were made from 1 slowly-adapting type I (SAI), 2 SAI and 3 fast-adapting type I (FAI) units via tungsten microelectrodes inserted percutaneously into the median nerve of awake human subjects. Vibrations was applied to the associated sensory receptor at 12Hz. Recordings were made in control conditions and with electrical noise applied externally proximal to the sensory receptor at 4 different amplitudes (15, 30, 45 and 60μA s.d.). In a second psychophysics experiment electrical noise of varying amplitude (0-120% of perception threshold) was applied to the index finger of 7 subjects while a vibration stimulus was applied to the finger pad. Results: We found that the application of subthreshold electrical noise can reduce the variance in interspike intervals by as much as 28%. Vibrotactile perception was improved in all subjects and the group experienced a significant improvement in perception (~10%, p < 0.05) with mid-level noise application (60 and 80% of perception threshold). Interestingly, the within-subject variance in vibration perception threshold was lower at these optimal noise levels. Conclusion: Microneurography and psychophysics data suggest that the noise-induced improvement in perception may be due to a reduction in spike timing variance. While this work is at an early stage it may have significant implications in the development of treatments for neurodegenerative conditions.

POS-MON-230

COCHLEAR IMPLANT USE CAUSES CHANGES IN THE COCHLEOTOPIC ORGANISATION OF AUDITORY CORTEX IN DEAF ANIMALS

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Neonatal deafness results in the loss of the normal cochleotopic organisation of primary auditory cortex (AI). Environmentally-derived chronic electrical stimulation, via a cochlear implant (CI), can restore that organisation. This study explores the time course of the changes in cochleotopic organisation by using chronic recordings of neural activity in auditory cortex of both normal hearing (NH, n=2) and neonatally deafened cats receiving auditory input via a CI (DCI, n=2). All animals had 6x10 ‘Utah planar silicon substrate recording electrode arrays (Blackrock Microsystems) implanted into putative AI as young adults. Multi-unit cortical responses to acoustic or electric stimulus were obtained under ketamine-xylazine anaesthesia at least monthly for approximately six months. In the NH animals, well characterised acoustic response areas were initially obtained on 67% of the recording channels, which decreased to 52% in the final recording session. In contrast, in DCI animals, well characterised electrical response areas were initially obtained on 22% of the recording channels, which increased to 69% of channels in the final recording session. There was no significant change in cochleotopic (tonotopic) organisation in NH animals over the 6-month recording period (paired t-test on characteristic frequency; p>0.1). However, DCI animals exhibited a significant change in cochleotopic organisation over the 6-month recording period (paired t-tests on best electrode; p<0.01). The first statistically significant changes in cortical organisation (paired t-tests; p<0.05) occurred after 4-5 months. These results demonstrate that auditory cortex can undergo changes in cochleotopic organisation (over several months) as a result of CI use. They also suggest chronic CI use increases cortical responsiveness.

POS-MON-231

THE FEASIBILITY OF EXPLANTING A SUPRACHOROIDAL ELECTRODE ARRAY IN A FELINE MODEL

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Purpose. To determine whether suprachoroidal electrode arrays can be safely removed and replaced after chronic implantation in a feline model. Method: Suprachoroidal electrode arrays were unilaterally implanted and surgically explanted (n=6), replaced (n=5) or left undisturbed (n=2) after one month. The retina was assessed pre and post-operatively using fundus photography and optical coherence tomography (OCT). Results: Three months post-implantation, electrode impedances were measured and the subjects were transcardially perfused. The eyes were prepared for histologic study. Results. Array explantation and replacement was successful in all subjects. OCT showed localized tapetal damage near the implantation site. Staphylomas developed post-implantation along the scleral incision site (n=5). Staphylomas developed near the implant's tip post-implantation (n=7). Staphylomas developed within the suprachoroidal space, retina and choroid. Conclusions. Cell-based therapies to facilitate brain repair represents a promising area of treatment for ischaemic stroke. We have isolated and characterized human neural progenitor cells (hNPCs) and differentiated them into a committed neuronal phenotype prior to transplantation. Purpose: To compare outcomes of undifferentiated and pre-differentiated hNPCs transplanted within the severely damaged rat brain with transplanted cells located outside the stroke affected area. Methods: The middle cerebral artery was constricted by endothelin-1 in conscious rats. 7 days after stroke undifferentiated, pre-differentiated hNPC’s or media alone were stereotaxically injected into the rat brain (n=10/group) at 8 predetermined sites to target both the damaged and non-damaged striatum and cortex. Brains were harvested 28 days post-transplant. Results: Immunohistochemical analysis revealed that both undifferentiated and pre-differentiated hNPCs survived the grafting procedure. Pre-differentiated hNPCs maintained their neuronal phenotype post-transplant as evidenced by human nuclear antigen (hNA) colabeled with βIII-tubulin, GABA, and Glial Fibrillary Acid Protein (GFAP). Ki67, Caspase or Tunnel stain. Pre-differentiated hNPCs were observed to be supported by neighboring blood vessels even in the core infarct, and transplants outside the infarct were observed to establish longer process than those within the core. Undifferentiated hNPCs were double-positive for hNA and GFAP indicating differentiation into astrocytes, and were mostly localized within the infarct border zone. Conclusion: Pre-differentiating hNPCs into neuronal cells prior to transplantation results in a greater number of hNPC-derived neuronal populations within the stroke-damaged brain. Neurite outgrowth and maturation appears dependent on the transplant environment and may require the presence of new blood vessels.

POS-MON-232

NEW BLOOD VESSELS SUPPORT TRANSPLANTED PRE-DIFFERENTIATED HUMAN NEURAL PROGENITOR CELLS IN THE STROKE DAMAGED BRAIN

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Cell-based therapies to facilitate brain repair represents a promising area of treatment for ischaemic stroke. We have isolated and characterized human neural progenitor cells (hNPCs) and differentiated them into a committed neuronal phenotype prior to transplantation. Purpose: To compare outcomes of undifferentiated and pre-differentiated hNPCs transplanted within the severely damaged rat brain with transplanted cells located outside the stroke affected area. Methods: The middle cerebral artery was constricted by endothelin-1 in conscious rats. 7 days after stroke undifferentiated, pre-differentiated hNPC’s or media alone were stereotaxically injected into the rat brain (n=10/group) at 8 predetermined sites to target both the damaged and non-damaged striatum and cortex. Brains were harvested 28 days post-transplant. Results: Immunohistochemical analysis revealed that both undifferentiated and pre-differentiated hNPCs survived the grafting procedure. Pre-differentiated hNPCs maintained their neuronal phenotype post-transplant as evidenced by human nuclear antigen (hNA) colabeled with βIII-tubulin, GABA, and Glial Fibrillary Acid Protein (GFAP). Ki67, Caspase or Tunnel stain. Pre-differentiated hNPC transplants were observed to be supported by neighboring blood vessels even in the core infarct, and transplants outside the infarct were observed to establish longer process than those within the core. Undifferentiated hNPCs were double-positive for hNA and GFAP indicating differentiation into astrocytes, and were mostly localized within the infarct border zone. Conclusion: Pre-differentiating hNPCs into neuronal cells prior to transplantation results in a greater number of hNPC-derived neuronal populations within the stroke-damaged brain. Neurite outgrowth and maturation appears dependent on the transplant environment and may require the presence of new blood vessels.
INTRODUCTION: Proof of principle for cell transplantation has been demonstrated for neurodegenerative diseases including Parkinson’s disease however extensive variability has been observed in both animal and clinical trials. In part, variability can be attributed to poor cell survival and neural network integration. We propose that an enhanced host environment capable of providing physical and trophic support for new cells may significantly improve this technology. Methods: We examined the survival, differentiation and neurite growth of primary neural stem cells/progenitors exposed to electrop spun poly-caprolactone (PCL), including tethered GDNF, in vitro and in vivo. In an effort to generate a material more suitable for implantation we produced a composite scaffold of PCL-short fibres embedded into a xylucogen gel, that was implanted into the brains of Parkinsonian mice, together with dopamine-encrusted ventral midbrain primary tissue. Results: We demonstrate that these scaffolds, in the presence of tethered GDNF, are capable of enhancing cell survival up to 2-fold in vitro, and 3-fold in vivo. These scaffolds additionally promoted neural stem cell proliferation (up to 10-fold); neural differentiation and neurite elongation. Conclusion: This study demonstrates that biofunctionalized scaffolds are capable of improving the in vitro and in vivo environment for neural stem/progenitor cells. The findings could have significant implications for improving cell replacement therapy for the treatment of neurodegenerative diseases.

INVESTIGATION OF METHAMPHETAMINE SELF-ADMINISTRATION IN RATS
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Purpose: The use and abuse of psychostimulant drugs represents a growing social and economic problem in New Zealand and Australia. Data from recent world drug reports list New Zealand and Australia as higher users of amphetamine-type stimulants than the rest of the world (UNODC, 2012). Amphetamine addition is a complex neurobiological problem; neuroadaptations that occur in response to drug use involve a number of protein changes, both in localisation and abundance. Addiction findings could have significant implications for improving cell replacement therapy for the treatment of neurodegenerative diseases.

COGNITIVE TRAINING IN THE WORKPLACE: WHO BENEFITS? AN INVESTIGATION OF ROLE TYPE AND TRAINING DURATION
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This study was conducted to identify those who would benefit most from cognitive training (CT) in the workplace, depending on the cognitive demands of the job performed. It also examined the effects of different doses of CT on cognitive enhancement and the interaction between cognitive capacity and training dose. This was a repeated measures, non-randomised, longitudinal, non-controlled, retrospective multi-centre study. De-identified data were provided to the researcher by an independent third party software company. Our sample of 270 white collar workers from a variety of Australian organisations was split according to those performing roles of lower cognitive complexity (support staff, frontline staff) versus more cognitively complex roles (technical specialists, managers and executives), as well as training time (classified as number of training sessions, duration of training, and training intensity, that is, number of sessions per day). Compliance with training was set at a minimum of 70%. Repeated measures ANOVAs were used to investigate effects of TIME by GROUP (cognitive complexity and training time). While trends for accuracy and response time were in expected directions across the entire cohort, that is, increased accuracy and reduced response time, there were no significant group effects for either cognitive complexity or training time. However, there was a significant 3-way interaction for overall Response Time, whereby those in less cognitively demanding roles benefited from more training sessions (p=0.02*) and training over a longer period (p=0.03*) than trainees in more cognitively complex roles. High Training Intensity improved Overall Cognition in those in cognitively complex jobs in contrast to those in jobs of low cognitive demand who benefited most from low intensity training (p=0.00***). The effects of job complexity and training time appear to confer selective benefits on trainees, which has important implications for the design and delivery of online CT in a work setting.

A NOVEL TECHNIQUE THAT COMBINES SINGLE CELL ELECTROPRODUCTION (SCE) WITH EXTRACELLULAR RECORDINGS IN VITRO AND IN VIVO
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Purpose: In SCE a pipette typically containing DNA solution, is placed into gentle contact with the cell membrane and electrical pulses are delivered through the pipette, temporarily permeabilising the cell membrane and driving DNA into the cell. The strength of this technique is that it permits genetic tools to be applied at single cell resolutions. Conventional SCE requires optical guidance, restricting target selection to anatomical, rather than functional, criteria. Here we describe a form of SCE compatible with single unit recordings in vivo. Methods & Results: Initially we adapted a standard recording amplifier to deliver trains of current pulses to a pipette containing fluorescent dextran, we demonstrated efficient SCE (37/48 cells) while maintaining cell viability. However, the success rate of SCE was inversely proportional to the distance between the pipette and target (4/10 and 1/11 cells at 3 and 6μm respectively). For use in vivo we therefore needed to establish an electrophysiological indicator of pipette contact. In vitro we found a strong positive predictor of SCE was a small transient pipette hyperpolarization in response to a single high amplitude current pulse (39/48 cells). In urethane-anaesthetised rats the same approach has been successful in cells recorded up to 4.6mm deep to the brain surface (18/32 cells). Conclusion: We have developed a technique that combines SCE with extracellular recording, negating the need for optical guidance and permitting functional identification of target neurons prior to electropermeabilisation. The approach will permit the application of novel genetic techniques to physiologically characterised neurons.
**POS-MON-237**

**3D MULTI-SITE PHOTOSTIMULATION WITH HIGH-SPEED SWITCHING**

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**Purpose:** Scanning techniques offer pseudo-simultaneous photostimulation but are restricted to a single optical plane. Holographic projection allows multi-site photostimulation in 3D but has limited switching capability that cannot match physiological timescales for analysing neurotransmitter activity. We combine holographic projection and high-speed spatial light switching to provide spatial-pattern flexibility and physiological temporal resolution of synaptic inputs. **Methods and Results:** Our setup includes a two-photon fluorescence microscope for rendering the 3D neuronal structure from which stimulation sites are chosen. A phase-only spatial light modulator encoded with the appropriate phase-hologram is used to project multiple stimulation foci at which highly localized two-photon uncaging of neurotransmitters is achieved. A programmable spatial amplitude modulator is used to switch individual focal spots at independently up to 1.4 kHz. The system was tested in vitro using 300μm thick slices of rat somatosensory cortex and hippocampus (P22-35). Whole-cell recordings of layer II/III, V and CA1 pyramidal cells were obtained. Neurons filled with 0.1 mM Alexa 488 were imaged at 780-800 nm. 3 mM MNI-glutamate was bath-applied and uncaged at 720 nm in the presence of 0.1 mM cyclothiazide. Using 15-30 mW power per uncaging spot, we were able to uncage at up to 8 simultaneous sites on several dendrites extending into different planes. Using complex spatio-temporal patterns of multi-site uncaging, we elicited action potentials. **Conclusion:** This technique has the capability to switch stimulation sites at physiological temporal resolution while allowing simultaneous uncaging at multiple 3D locations on dendritic trees. It offers unprecedented flexibility in designing spatio-temporal input patterns for synaptic integration studies.

**POS-MON-238**

**QUANTITATION OF NEUROTRANSMITTERS AND METABOLITES IN MICORDIALYSATE FLUID AND BRAIN USING ADVANCED GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)**

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**Purpose:** Sensitive and reliable measurement of neurotransmitters and other metabolites in brain is important for examining changes in brain biochemistry and metabolism. Our aim was to develop reliable analytical protocols for accurate measurement of different neurotransmitters and metabolites in cerebral spinal fluid (CSF) collected via microdialysis, focusing initially on several amino acids relevant for neurotransmission. The effect of potassium stimulation as a physiological control on extracellular levels of these compounds was also investigated. **Method:** CSF samples from the hippocampus and prefrontal cortex of male C57BL/6 mice (n=6 per region) were collected via microdialysis (flow rate 1 μl/min), before and after an infusion of artificial CSF containing 50 mM K\(^+.\) Following heavy isotopic dilution, metabolite extraction and derivatization, samples were analysed using triple quadrupole GC-MS and quantified using calibration standards. **Results:** 10 μl CSF dialysate was sufficient for accurate measurement of several neurotransmitters and several metabolites including GABA (mean 40 nM ±6 SEM), glutamate (45 nM ±4), alanine, dopamine, glycine and serine. Stimulation with 50 mM K\(^+\) induced a significant 2-3-fold increase of alanine, GABA and glycine (p<0.05). **Conclusions:** GC-MS targeted analysis provides a sensitive tool for measuring a large range of neurotransmitters and metabolites during a single analysis in small aliquots of CSF. Small amounts of brain, plasma and other tissues can also be analysed. We are currently monitoring metabolic changes in a transgenic R6/1 mouse model of Huntington's disease to examine mechanisms of neurodegeneration and identify potential biomarkers.

**POS-MON-239**

**INDIVIDUALLY VENTILATED CAGE SYSTEMS: A NOVEL HOUSING TYPE CAUSING PROBLEMS FOR MOUSE MODEL RESEARCH**

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**Purpose:** Over recent years, the use of individually ventilated cage (IVC) rack systems in laboratory mouse facilities has increased. Compared to conventional housing, these IVC rack systems provide a very different environment, as cage structures are limited as is external acoustic and olfactory stimulation. The frequency of stressful cage changes is also reduced. Importantly, studies so far have concentrated on the evaluation of behavioural effects of IVC rack systems on wild-type (WT) mice. Here we present the first data on how different cage systems (IVC versus traditional) impact on the behavioural phenotype of a mouse mutant for the schizophrenia candidate gene neuregulin 1 (Nrg1 HET mice).

**Methods:** Male and female Nrg1 HET mice and their WT littermates (all on pure C57BL/6J background) were bred and raised until postnatal day 90 in either IVC or traditional cage systems. Mice (N=12/cohort) were then tested in a comprehensive battery test for locomotion, exploration, anxiety, social interaction, cognition and sensorimotor gating. Results: IVC systems suppressed the hyper-locomotive phenotype typical for Nrg1 HET mice kept in traditional cage systems. Furthermore, impairments in fear-associated contextual learning of Nrg1 hypomorphs were modified by IVC housing and sensorimotor gating deficits of Nrg1 HET mice were absent when animals were kept in IVC. Finally, mice in IVC appeared more sensitive to the locomotor-stimulating effects of an acute challenge with 0.25 mg MK-801. Some of the behavioural effects described showed sex-specific characteristics. **Conclusions:** Our data reveal for the first time the significant impact of IVC systems on genetic mouse models for brain disorders. Thus, researchers have to consider the breeding and housing conditions of their test cohorts carefully and consider housing condition differences, when comparing data across institutions.

**POS-MON-240**

**UBIQUITINATION AND TRANSPORT OF PTEN IN CELLS REVEALED BY BIMOLECULAR-FLUORESCENCE COMPLEMENTATION**

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**Background:** The division and growth of cells in the brain is driven by the Akt/MTOR pathway that is negatively regulated by PTEN. Loss of PTEN can result in macroencephaly and tumours due excessive PI3-kinase signalling. PTEN is known to have different functions depending on its location inside the cell; Therefore the transport of PTEN is important for determining PTEN activity. We recently identified that the ubiquitination of PTEN is mediated by the adaptor protein Ndfip1. However, the movement of PTEN inside the cell following ubiquitination is unclear. Here we investigated in real time the ubiquitination and transport of PTEN using bimolecular-fluorescence complementation (BiFC) imaging. **Methods:** Interactions between Ndfip1 and PTEN, as well as PTEN and ubiquitin were investigated using BiFC. **Results:** Ubiquitinated PTEN localized mainly in peri-nuclear and nuclear regions. 2) Ubiquitinated PTEN is co-localized with early and recycling endosomes, but not late endosomes. 3) Ndfip1 binds with PTEN in the cytoplasm, co-localizing with early and recycling endosomes. 4) Rab5, which regulates early endosome function was found to be important for PTEN ubiquitination and trafficking. **Conclusion:** Ubiquitinated PTEN was found to be transported to nuclear and peri-nuclear locations. The endosomal pathway is critical for this transport as disruption of Rab5 inhibits ubiquitination and trafficking. These findings increase our understanding of PTEN trafficking and how it contributes to neoplasia and neuronal survival.
POS-MON-243

EFFECTS OF REDUCED RADIATION DOSE ON GENE EXPRESSION CHANGES IN CULTURED MURINE BRAIN ENDOTHELIAL CELLS

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Purpose: Brain Arteriovenous Malformations (AVMs) are abnormal connections between arteries and veins and are the leading cause of hemorrhagic stroke in young adults. Safe and effective treatment of large and deep AVMs is often not possible, therefore new treatment methods are required. A possible new treatment involves using stereotactic radiosurgery to selectively alter the AVM endothelial phenotype, allowing radiation to selectively ablative the AVM endothelial cell phenotype.

Methods: Murine brain endothelial cell cultures were treated with 15Gy (n=4) and 25Gy (n=4) using a linear accelerator. Non-irradiated cell cultures were used as controls (n=4). Quantitative PCR was used to measure the relative gene expression at 6, 24, 48 and 72h after radiation. Results: Genes encoding for integrin, adhesion and migration were significantly up-regulated in both ICAM-1 and VCAM-1 expression. Conclusions: Radiation alters gene expression in the murine brain endothelial cell phenotype.

POS-MON-244

DEVELOPMENT OF SUBCELLULAR FRACTIONATION TECHNIQUES TO DETERMINE THE INTRACELLULAR COBALAMIN TRANSIT IN VITRO AND THE IMPACT OF LYSOSOMAL DYSFUNCTION

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Intracellular cobalamin (Cbl) utilization is critically dependent on its efficient transit through the lysosome and subsequent delivery to cytosol and mitochondria. We propose that age-related pathological processes may inhibit lysosomal function and impair intracellular Cbl transport.

Purpose: To investigate alterations of intracellular [57Co]Cbl trafficking in subcellular organelles when lysosome function is interrupted it is essential to develop an optimal subcellular fractionation method to isolate pure lysosomes, mitochondria and cytosol.

Methods: Approximately 100 million human SH-SY5Y neurons or HT1080 fibroblasts were labelled with [57Co]Cbl, homogenised using a ball-bearing homogeniser and the lysates fractionated using an Optiprep gradient and reagent kits from either Pierce or Sigma. [57Co] in each fraction was measured using a gamma counter and subcellular fractions were probed by western blotting.

Results: Both protocols separated subcellular organelles to a certain extent. The Pierce method seemed to be superior, separating pure lysosomes from mitochondrial fractions without cytosol contamination. Similar results were obtained using HT1080 fibroblasts.

Conclusion: Development of subcellular fractionation methods provides a useful tool for investigating intracellular Cbl trafficking. This method can be adapted to study the impact of age- or pathology-related lysosomal dysfunction on intracellular [57Co]Cbl transport.
**POST-MON-245**

**IMPROVED GC-MS METHODS FOR ANALYSIS OF HOMOCYSTEINE, METHIONINE AND METHYLMALONATE IN CULTURED CELLS**

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**Purpose:** The aim of the present study is to establish a new gas chromatography mass spectrometry (GC-MS) method using a triple quadrupole which would allow for more sensitive quantification of changes in homocysteine, methionine and methylmalonate in small numbers of cultured cells and brain tissue. Homocysteine, methionine and methylmalonate are established markers of cobalamin deficiency.

**Methods:** Cultured cells (1 x 10^6) and 5mg of human brain were incubated with 10 mg/ml dithiothreitol in methanol for one hour at room temperature to reduce homocysteine disulfide bonds. Lipids were removed using methyl-tet-butyl ether and the aqueous phase derivatized with N-Methyl-N-tet-butyldimethylsilyl trifluoroacetamide (MTBSTFA). Samples were subsequently analysed with a 7000 Agilent triple-quadrupole GC-MS. Multiple reaction monitoring was performed on the compounds in electron ionisation mode at 70eV. Corresponding deuterated internal standards were used to quantify compounds. **Results:** We identified the following transitions: 420->392, 318->292, 322->292, 218, 323->295, 221, 289->189, 147 292->192, which allowed for sensitive quantification of homocysteine, methionine and methylmalonate and their deuterated standards respectively. The lowest limits of detection for homocysteine, methionine and methylmalonate were found to be 0.001 pM in three different cell lines and brain tissue. Sample preparation and analysis was complete in 4 h. **Conclusion:** Homocysteine, methionine and methylmalonate were successfully quantified in low cell numbers. This method is widely applicable for studies of cobalamin function in cells and has the potential advantage of conserving precious human tissue samples.

**POST-MON-246**

**INFERENCE OF MECHANICAL STATES OF PERISTALIS BASED ON HIDDEN MARKOV MODELS**

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**Purpose:** Defining the relationships between gut wall motion, intraluminal pressure and flow of content is critical for understanding normal and abnormal gut motility. From recorded changes in gut diameter (video) and intraluminal pressure (manometry) software, developed in Matlab, was written to define discrete functional mechanical states during myogenic and neurogenic activity in rabbit colon. **Methods/Results:** Simultaneous pressure and diameter were recorded from isolated rabbit distal colon kept in oxygenated Krebs solution. Spatiotemporal maps of combined pressure and diameter were constructed. From these combined maps dynamic states were distinguished as increasing, decreasing and stable states. Two additional static states of gut diameter were defined, corresponding to fully occluded and fully distended. The functional mechanical states of the muscle were defined as combinations of these five factor states. A hidden Markov model was used to model the probability distribution of diameter and pressure values and their time derivatives, on the assumption that the muscle must be in any one of the possible mechanical states. The Viterbi algorithm was used to infer the most likely sequence of mechanical states based on the observed data. Quiescence was modelled with a normal distribution at 0, with variance being a free parameter. The probability density function of non-quiescent factor states was modeled with the normalized cumulative density function of the normal distribution, with equal variance. From this a spatiotemporal map of the mechanical states was produced, showing the contractile activity of each 1cm segment of the gut throughout the entire period of recording. **Conclusion:** Inference in the hidden Markov model achieves noise-reduction, segmentation, and classification of observed changes in diameter and pressure values into well-defined functional mechanical states.

**POST-MON-247**

**SHOOTIN1 ACTS IN CONCERT WITH KIF20B TO PROMOTE POLARIZATION OF MIGRATING NEURONS**

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Shootin1 has been ascribed a role in regulating polarization of primary hippocampal neurons. Shootin1 is also highly expressed in the developing cortex, prompting us the investigation of its potential role in polarization of migrating cortical neurons. Shootin1 knockdown impaired migration and polarization of cortical neurons. Biochemical assays performed after repeated cycles of microtubule polymerization-depolymerization indicated that Shootin1 became enriched in the microtubule-associated protein fraction but did not bind microtubules directly. We also identified a member of the kinesin superfamily, KIF20B, a known microtubule-associated molecular motor, as a novel Shootin1 interacting protein and potential mediator of Shootin1 interaction with microtubules. KIF20B/Shootin1 binding was mapped to a 57 amino acid KIF20B sequence. Direct interaction between that peptide and Shootin1 was confirmed by surface plasmon resonance-based technology and binding affinities were calculated. These proteins also formed a complex in vivo based on co-immunoprecipitation experiments. In primary hippocampal neurons KIF20B knockdown reduced Shootin1 mobilization to the developing axon, as evidenced by immunostaining and fluorescence recovery after photobleaching (FRAP) analysis, suggesting that Shootin1 is a novel KIF20B cargo. shRNA targeting of Shootin1 reduced PIP3 accumulation in the growth cone, as did KIF20B shRNA. In the developing mouse brain, KIF20b knockdown or expression of the KIF20B minimal binding domain inhibited neuronal migration, and in vivo migration assays suggested that Shootin1/KIF20B act in the same genetic pathway. Time-lapse imaging of multipolar cells in the SVZ revealed that downregulating levels of either Shootin1 or KIF20b hindered the transition from multipolar to bipolar cells. Collectively, our data demonstrate the importance of the Shootin1/KIF20B interaction to the dynamic process of pyramidal neuronal polarization and migration.

**POST-MON-248**

**TUBB5 REGULATES CEREBRAL CORTICAL DEVELOPMENT AND ITS PATHOGENIC MUTATIONS DISRUPT NEURONAL DIFFERENTIATION**

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During cerebral cortical development, the migration of newborn neurons to their appropriate position is a critical step which influences their proper maturation and precise connectivity, all of which contributes to normal brain function. Recently, our preliminary data has indicated that deletion of the cytoskeletal gene tubb5 by RNAi within the embryonic mouse cortex disrupted cell migration in vivo. Furthermore, three de novo missense mutations were identified in unrelated human subjects diagnosed with microcephaly as well as structural brain disorders, suggesting that TUBB5 was important for normal brain development. **Purpose:** However, given the prominent expression of tubb5 in progenitor cells and postmitotic neurons of the embryonic cortex, we sought to determine if the migration of postmitotic neurons was directly affected by tubb5 deficiency; as well as to determine whether the three TUBB5 missense mutations were pathogenic. **Methods/Results:** Using an expression vector to specifically label postmitotic neurons, we found that knockdown of tubb5 resulted in their defective migration within the E17 mouse cortex (n=3). Interestingly, the morphologies of tubb5-deficient cells were also altered when compared with control, suggesting a potential correlation between their defective migration and abnormal shape acquisition. Furthermore, overexpression of each of the three missense mutations of TUBB5 significantly disrupted cell migration within the E17 mouse brain (n=5 per condition). **Conclusion:** Our results demonstrate that tubb5 has important functions for regulating the migration of postmitotic neurons during cortical development, and that the three TUBB5 missense mutations disrupt normal brain development.
POS-MON-249

PROBING THE ACTION OF NEURONAL JNKS: IDENTIFICATION AND CHARACTERIZATION OF THE BRAIN JNK1 AND JNK3 INTERACTOME

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The c-Jun N-terminal kinases (JNKs) are a subfamily of mitogen-activated protein kinases, when activated in response to stresses such as ischemia and reperfusion, might pathologically phosphorylate various substrates such as c-Jun and ATF2. JNKs are encoded by three genes; Jnk1, Jnk2, and Jnk3. The paper aimed to identify these pathologically affected substrates as well as explore the functional significance of JNK1/JNK3 interactionome of the brain that will drive future research on the pathological significance of JNKs.

POS-MON-250

ASSESSING THE FUNCTIONAL SIGNIFICANCE OF THE HORIZONTAL EFFECT

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Humans have been shown to have poor sensitivity to horizontally oriented stimuli containing broad spatial frequency and orientation content. This has been called the Horizontal Effect and it is proposed that it arises from an interaction between anatomical anisotropies in selectivity of cells and gain control mechanisms in the early visual system. Purpose: These findings have been reported with limited sample sizes (n=6), yet individual visual factors could influence the presence of the horizontal effect and may shed further light on the functional significance of the effect. The aim of the current study was to assess the generality of the Horizontal Effect in the population. Methods: This study replicates work by Essock and colleagues (Essock, DeFord, Hansen, & Sinai, 2003; Essock, Hauen, & Kim, 2009; Hauen & Essock, 2010), with a greater number of participants (n=30). Participants were asked to identify an oriented pattern from noise in a 2IFC detection task. Both target and noise stimuli contain 1/4 spatial frequency content. While the noise stimulus was constructed using an isotropic orientation filter target stimuli were constructed using a triangular filter with either ±2° or 0° half-widths. 4 orientations were tested; vertical, horizontal, and the two obliques. Results: While we verify the existence of a Horizontal Effect in some participants, 5/30, it is not consistent across observers and no consistent pattern of effects was found. Conclusions: This finding calls for a critical revaluation of the functional significance of perceptual anisotropies generated by broadband stimuli.

POS-MON-251

KNEE JOINTS SIGNIFICANTLY AFFECT ESTIMATION OF BODY KINEMATICS IN THE SAGITTAL PLANE DURING STANDING QUIETLY

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Background: Upright stance control has been recently modeled as a double-link inverted pendulum including motion of hip and ankle joints. Although the model assumes knee joint motion is negligible, a few studies pointed the motion contributed to postural control in upright stance. Effects of knee joint motion on the body kinematics should be described. Purpose: This study aimed to investigate behavioral effect of the knee joint motion on the body kinematics during quiet stance. Methodology: Nine healthy adults were asked to stand quietly for 30s on a force platform. Six optical motion cameras captured angular motions of ankle, knee and hip joints in the sagittal plane. Angular accelerations of the joints obtained by double differentiation of the angular displacements were embodied into three models, single-(SIP, ankle only), double-(DIP, ankle and hip), and triple-link inverted pendulum (TIP, ankle, knee, and hip) models to estimate acceleration of the anterior-posterior (A-P) center of mass (COM). Actual COM acceleration (ACT) was obtained from A-P ground reaction force divided by a subject’s body mass. Amplitudes of estimated and actual COM accelerations were compared by a one-way ANOVA with a Tukey-Kramer post hoc test. Result: Both the SIP and DIP models significantly overestimated the COM accelerations (p-values<0.05 ). By contrast, the COM acceleration estimated by the TIP model showed good coincidence with the ACT. Conclusion: The results showed knee joint sufficiently affected the whole body COM. This implies the TIP model can describe the COM in upright stance more accurately than the SIP and DIP models.

POS-MON-252

EYA AND SIX FAMILY MEMBERS REGULATE MYOGENIC STEM CELL FATE

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Purpose: Satellite cells are the primary stem cell responsible for growth, maintenance and repair of skeletal muscle, and as such are a viable cell source for the therapeutic treatment of muscular dystrophies. Satellite cells live a complex life; they lie dormant in stable muscle, however when repair is required, satellite cells activate and produce sufficient progeny to differentiate effecting repair, and then return themselves to quiescence, poised for next time. This requires an exemplary capacity to respond appropriately to signalling. During development, myoblasts express a suite of transcription factors that are critical in deciding their fate – when to proliferate, when to differentiate, when to move, when to sit still, and when to give up and die. The exquisite, fine regulation of cell fate decisions in muscle satellite cells requires a highly adaptive platform of gene expression. We have explored the function of some major players responsible for modifying the satellite cell transcriptional landscape during activation, proliferation and myogenic differentiation. Methods: We have investigated the role of Pax, Six, and Eya family members in controlling the transcriptional landscape of satellite cells using retroviral expression, dominant negative constructs, siRNA-mediated knockdown, immunocytochemistry, RT-qPCR, chromatin-immunoprecipitation, microarray and next-generation sequencing. Results: We have defined members from each gene family that participate in regulating critical functions of satellite cells during in vitro myogenesis. Conclusion: This research provides an in-depth analysis of transcription factor function in a model stem cell. This approach has uncovered interesting new players in satellite cell biology, and revealed some new tricks for an old dog.
CONSERVED DETERMINANTS OF PAIN PERCEPTION

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Acute and chronic pain will affect most people at some stage in their lives, and chronic pain in particular represents an unmet clinical need. Nociception, the neuronal sensory and processing apparatus that relays the perception of acute pain, allows an organism to evade potential tissue damage and death, and many genes regulating this process may be conserved across phyla. Using a genome-wide neuronal-specific RNAi knock-down strategy in adult Drosophila, we performed the first neural-specific global screen for an innate behavior, and identified hundreds of novel genes required for perceiving and avoidingnoxious heat. Based on this data, we have constructed an evolutionary-conserved, global network map, identifying molecular components and key pathways involved in thermal pain perception. Work in fly, mouse, and human systems have helped us to confirm many novel Drosophila nociception genes as bona fide conserved "pain" genes, and our recent progress will be presented.

PRODUCTION OF IMMATURE NEURONS IN ADULT RAT HIPPOCAMPUS IS STIMULATED BY DIFFUSE TBI WITH HYPOXIA, AND THIS EFFECT IS PROLONGED BY TREATMENT WITH ERYTHROPOIETIN

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Purpose: Diffuse injury is a common feature of traumatic brain injury (TBI), and combined with hypoxia, worsens outcome. Following focal TBI, stimulation of neurogenesis is associated with improved outcome, while manipulating neurogenesis in diffuse TBI remains unexplored. We characterised neurogenesis in a combined diffuse TBI/hypoxic insult model, then attempted to augment neurogenesis by treatment with erythropoietin (EPO). Methods: Traumatic axonal injury (TAI) was induced by dropping 450g weight from 2m, and hypoxia (Hx) by 14% C2 ventilation for 30min. Experimental groups included Sham, Hx, TAI and TAI+Hx. Rats were administered rhEPO-alpha (5000IU/kg) or vehicle at 1and24h after injury, and BrdU was administered d1-3 (150mg/kg). Brains were collected at 7 and 14d for immunohistochemical analysis of phospho-Erk (MEK) and neuronal differentiation (Dcx) in the hippocampal dentate gyrus (DG) (n=3-6). Results: Numbers of BrdU+ cells were not increased in any injury group at 7 or 14d. However, TAI+Hx led to a 75% increase in Dcx+ cells at 7d (p<0.001). Increased production of immature neurons was transient, with sham levels at 14d post-TAI+Hx. EPO did not affect proliferation at either time-point in any group, and did not increase immature neurons at 7d. However, 14d post-injury, EPO increased Dcx+ cells in TAI and TAI+Hx groups (p<0.001). Conclusion: These data demonstrate that the more severe injury of combined diffuse TBI with hypoxia leads to greater stimulation of neurogenesis than either alone. Furthermore, EPO treatment leads to prolonged production of immature hippocampal neurons, potentially contributing to its demonstrated capacity to improve cognitive recovery after TBI.

HUMAN DENTAL PULP STEM CELLS REDUCE CORTICAL PERINEURONAL NET EXPRESSION IN VITRO

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Purpose: The Perineuronal net (PNN) is a specialised extracellular matrix that provides important structural and functional supportive roles in the healthy central nervous system (CNS). However, following CNS injury there is upregulation of the PNN in damaged areas that may restrict recovery by forming an inhibitory peri-injury region. In the rodent stroke brain treatment with human adult Dental Pulp Stem Cells (DPSC) has been shown to enhance functional recovery. We hypothesized that benefit was induced by paracrine factors secreted by DPSC that positively influence neuroplasticity post-stroke. This may relate to stem cells regulating the PNN. The aim of this study was to investigate in vitro whether the co-culture of DPSC with cortical neural cells may downregulate the expression of the PNN. Methods: Cortical neural cells were isolated from postnatal day 1 C57 Black mice and cultured for 14 days to allow the development of the PNN. We co-cultured a range of cell dosages using 1000, 2000 and 5000 murine and human DPSC per cm², in comparison to human foreskin fibroblast cells (HF) for 24 hours. Expression analysis of the PNN was undertaken using a well characterized immunohistochemical stain for Wisteria Floribunda Agglutinin (WFA) co-localised to NeuN-positive neurons. Results: Co-cultures with 2000 and 5000 human DPSC per cm² resulted in a significantly lower proportion of NeuN-positive cells that co-expressed WFA. Murine DPSC and HF co-cultures did not show a significant change in WFA expression. Three individual human DPSC populations were found to show similar results. Conclusion: This is the first study, to our knowledge, to demonstrate human stem cell treatment can downregulate the PNN expression in cortical tissue.

POTENTIAL OF ALPHA-LIPOIC ACID (ALA) ON ARSENIC INDUCED CELL LOSS IN DEVELOPING RAT HIPPOCAMPUS

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Purpose: Exposure to Arsenic during developmental period interrupts the maturation of the central nervous system to cause neurobehavioral deficits, lower IQ performance and cognitive impairment. The present study was designed to determine the potential role of ALA on rat hippocampus following exposure to NaAsO2 during early postnatal development. Methods: Mother reared Wistar rat pups were randomly assigned into control and the experimental groups. The experimental groups received NaAsO2 alone (1.5 & 2.0 mg/kg body wt) or along with ALA (70 mg/kg body wt) by intraperitoneal route from postnatal day (PND) 4 to 15. Morris water maze testing (n=8) was performed for 5 consecutive days (PND 14-18) for assessment of learning and memory. On PND 16, animals (n=6) were sacrificed by cervical dislocation and hippocampus was dissected out and used for quantification of apoptotic proteins Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic) by western blot analysis. On PND 16 animals were perfusion fixed with 4% paraformaldehyde and brains obtained were processed for paraffin embedding (n=5) and Cryo processing (n=5). Serial sections (7µm) were cut (Bregma -3.2 mm to 3.8 mm) from paraffin embedded blocks and processed for TUNEL staining while 30 µm thick cryosections were processed for immuno staining of Bcl-2 and Bax proteins and analyzed. Golgi - Cox staining was done to analyze the dendritic morphology. Results: The findings obtained demonstrate significant alteration in learning and memory due to arsenic exposure. Arsenic induced neuronal loss and altered dendritic morphology in rat hippocampus was confirmed by the TUNEL and immuno staining followed by the western blot analysis and Golgi analysis. Exogenous supplementation of ALA with arsenic showed significant improvement in learning & memory and decreased neuronal cell death. Conclusion: These findings indicate potential role of exogenous ALA on arsenic induced adverse effects on developing rat hippocampus.
such as schizophrenia and bipolar disorder, which are associated with Abnormal expression of NCAM in humans may thus induce disruptions.

NCAM-mediated adhesion resulted in reduced synaptic vesicle recycling. linked to the synaptic vesicle recycling machinery. Acute disruption of that NCAM associates with CASK and AP2, and is therefore directly 

synaptosomes isolated from mouse brain (n=3 experiments) showed 

accumulates in the presynaptic boutons of active excitatory glutamatergic synapses. Abnormalities in NCAM2-mediated synaptic 

mediated adhesion in cultured hippocampal neurons results in changes in dendrite morphology and number and molecular composition of glutamatergic synapses. Abnormalities in NCAM2-mediated synaptic adhesion may thus contribute to the mental disorders associated with mutations in NCAM2 gene.

POS-MON-257

DEHYDROEPIANDROSTERONE PRODUCTION IN THE BRAIN OF THE DEVELOPING SPINY MOUSE

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Purpose: Dehydroepiandrosterone (DHEA) is a multifunctional androgen which has a variety of actions in the CNS[1], including trophic effects on neuronal growth and differentiation [2]. Perhaps significantly for the developing brain, DHEA also protects neurons against glucocorticoid-induced neurotoxicity [3-5] and other perturbations that result in oxidative stress [7]. We have recently found that the adrenal gland of the spiny mouse (Acomys cahirinus), is capable of synthesising and releasing DHEA, together with the glucocorticoid cortisol. This finding led us to investigate if DHEA could also be produced de novo in the developing brain of this species.

Methods: Expression of the enzyme and accessory protein responsible for the synthesis of DHEA, 17-hydroxylase and 17-20 lase (P450c17), and cytochrome b5 (cyt b5), were determined in fetal (35 days of 39 day gestation), neonatal (day of birth) and adult (160 days postnatal age) brains by immunocytochemistry. The cellular location of P450c17 and cyt b5 were investigated by double-label immunofluorescence with neurons (NeuN), astrocytes (GFAP) and oligodendrocytes (CNPase). The bioactivity of the P450c17 enzyme was evaluated by the ability of fetal, neonatal and adult brain slices to convert pregnenolone (PREG) precursor into DHEA, as determined by radioimmunoassay.

Results: During fetal and early neonatal life P450c17 and cyt b5 were diffusely expressed in the white matter tracts of the pons and medulla, as well as in synaptic boutons in the brain stem. P450c17 was also seen in the pontine nuclei, and co-expressed with neurons in the midbrain. In the adult, P450c17 was co-expressed only with CNPase, and was found in the motor nucleus of the trigeminal nerve, in the striatum, and in the corpus callosum. Culture of explant fetal brain slices in the presence of 2.5mM PREG resulted in a maximum synthesis and secretion of 22.4±2.00ng DHEA/mg of tissue after 48 h in culture, which was significantly higher (p<0.01) than the synthesis of 0.04±2.04ng DHEA/mg of tissue in the adult over 48 h. Conclusion: This study shows that the fetal spiny mouse brain is able to synthesise and secrete DHEA in significantly higher amounts than the adult brain, and furthermore, there is differential expression of the enzymes necessary for the synthesis of this important neuromodulator throughout life. Since DHEA is neuroprotective and important for brain development, these observations suggest the spiny mouse may be a good model in which to study neurobehavioral pathologies reported to arise in children after illness and stress during pregnancy.

POS-MON-258

NCAM2-MEDIATED SYNAPTIC ADHESION IN THE MAINTENANCE OF GLUTAMATERGIC SYNAPSES

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The neural cell adhesion 2 (NCAM2) belongs to the immunoglobulin superfamily of cell adhesion molecules, which plays an important role in neuronal development and formation and maintenance of synapses. NCAM2 gene is triplicated in Down syndrome, and genome wide association studies implicate NCAM2 in mental disorders. Little is known however about the functions of NCAM2 in neurons. We show that NCAM2 is a synaptic cell adhesion molecule accumulating at the excitatory glutamatergic synapses. Synaptic levels of NCAM2 increase in response to the induction of long term potentiation in cultured hippocampal neurons (n=3 independent experiments) suggesting that it plays a role in learning and memory formation. In synapses, NCAM2 forms a complex with the glutamate receptors of AMPA and NMDA subtypes and a scaffolding protein Shank. Acute disruption of NCAM2-mediated adhesion in cultured hippocampal neurons results in changes in dendrite morphology and number and molecular composition of glutamatergic synapses. Abnormalities in NCAM2-mediated synaptic adhesion may thus contribute to the mental disorders associated with mutations in NCAM2 gene.

POS-MON-259

THE NEURAL CELL ADHESION MOLECULE (NCAM) REGULATES SYNAPTIC VESICLE RECYCLING IN CNS SYNAPSES

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The neural cell adhesion molecule NCAM is a member of the immunoglobulin superfamily of cell adhesion molecules. In the central nervous system (CNS), NCAM accumulates in the pre- and postsynaptic membrane of synapses. Our previous studies showed that NCAM plays an important role in the assembly of the postsynaptic signalling complex playing an important role in memory formation (Sytnyk et al., 2006). Functions of NCAM in the presynaptic membrane of the CNS synapses remained, however, poorly understood. We show that NCAM accumulates in the presynaptic boutons of active excitatory glutamatergic synapses. Western blot analysis of NCAM immunoprecipitates from synaptosomes isolated from mouse brain (n=3 experiments) showed that NCAM associates with CASK and AP2, and is therefore directly linked to the synaptic vesicle recycling machinery. Acute disruption of NCAM-mediated adhesion resulted in reduced synaptic vesicle recycling. Analysis of NCAM in humans may thus induce disruptions in neurotransmitter release and contribute to neurological conditions, such as schizophrenia and bipolar disorder, which are associated with mutations in NCAM.

POS-MON-260

BEHAVIOURAL ANALYSIS OF AGE-RELATED BALANCE DEFICITS IN MICE

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Discipline of biomedical Science, Sydney Medical School and Bosch Institute, University of Sydney, Sydney, NSW 2006.

Purpose: Ageing is commonly associated with deteriorating sense of balance. Despite this, we know very little about the behavioural or physiological decline that occurs with advancing age in any animal. Here we assessed the balance phenotype of mice using a barrage of behavioural tests and assessed the vestibular component of age-related decline in balance performance. Methods: All experiments outlined below were approved by the Animal Care and Ethics Committee at the University of Sydney. The balance phenotype of young (1 month) and older (>6 month) old C57/Bl6 mice was assessed using a standard Accelerating Rotarod (ARR) protocol. Briefly, a drum was accelerated from rest and measurements of time to failure (fall; TTF) were made from 4-5 trials. Mice were also trained to traverse a balance beam 60 cm long and their time to start (TTS), and time to traverse (TTT) were measured before and after a vestibular challenge- 20s rotation at 5 Hz in a custom-built rotator. Results: Using the ARR protocol the TTF for 8-9 month-old mice was shorter than that of 1-month-old mice (14.2 ± 3.3; n=15; vs. 23.4 ± 4.1s; n = 13; p = 0.00051). In addition older mice took significantly longer to traverse the balance beam when compared with young mice (5.48 ± 1.2s; n=11, vs. 3.53 ± 0.65s; n = 9; p = 0.002). Further, when the vestibular system of older mice was challenged they were more reluctant to begin the task than young mice (TTS; p = 0.037). Conclusion: Advancing age impairs the performance of mice on standard motor and balance tests. This change may reflect the time taken to re-equilibrate following vestibular challenge.
POS-MON-261

OLFACATORY ENSEATHING CELLS PHAGOCYTOSE AXONAL DEBRIS AND BACTERIA: IMPLICATIONS IN NEURAL REPAIR AND PROTECTION AGAINST MICROORGANISMS

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Purpose: Olfactory ensheathing glial cells (OECs) are essential for the continuous regeneration of the olfactory nervous system that occurs throughout life. Transplanted OECs have been trialled for neural repair with promising but variable results. The main challenge in the field is to elucidate the normal biological functions of these cells. We have characterised a biological function of OECs that has been largely neglected: the ability to phagocytose both debris from dead axons and bacteria. Methods: We used transgenic mouse lines in which olfactory neurons and glia express bright fluorescent proteins to visualise how OECs (red) phagocytose debris resulting from turnover of olfactory neurons (green) in vivo, in vitro and in tissue explants using a variety of advanced microscopy techniques. We also challenged cultured OECs with bacteria and studied their response. Results: Using confocal microscopy, we demonstrated that OECs contained large amounts of axon-derived debris both in vivo and in tissue explants. We also showed that OECs, and not macrophages, were the only phagocytic cells in immediate contact with olfactory axons. In response to axon-derived debris, OECs extended pseudopodia resembling those on activated macrophages (n=20). Using time-lapse imaging, we showed that OECs took up axonal debris primarily in cellular regions that extended highly motile lamellipodia (n = 5). Following large-scale degeneration of olfactory neurons by injection of methylazole, OECs had significantly more axonal debris (30-50% more, n=5, p<0.005) compared to controls. Furthermore, we demonstrated that cultured OECs were capable of phagocytosing bacteria (E. coli) in vitro. Conclusion: These results strongly suggest that OECs are the cells responsible for maintaining a clean, growth-promoting environment, thus allowing continuous regeneration of the primary olfactory nervous system. Our data also suggest that OECs may actively protect against microorganism invasion of the central nervous system via the olfactory nerve.

POS-MON-262

HISTOLOGICAL AND ULTRASTRUCTURE PATHOLOGICAL STUDY ON HIPPOCAMPUS FROM MESIAL TEMPORAL LOBE EPILEPSY

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Purpose: Mesial temporal lobe epilepsy (MTLE) is a kind of intractable epilepsy originated from mesial temporal sclerosis (MTS). It is generally considered that epilepsy derived from abnormal high synchronization of neurons, and anti-epilepsy durgs (AEDs) mainly improve neuron function. However, MTLE displays obviously resistance to AEDs, and can be effectively treated by surgery. The fact suggested non-neuron mechanism may underlay the development of MTLE. In this study we focus on changes of astrocytes in hippocampus tissues from MTLE patients underwent hippocampus resection. Method: A total of 29 hippocampus tissues from patients with temporal lobe epilepsy underwent surgery at Beijing Tian-Tan Hospital was included in this study. Astrocyte hyperplasia and it correlation with vascular were observed by HE staining, GFAP immunoflurescence, and transmission electron microscopy. Results: According to histological findings, hippocampus tissues were divided into non-mesial temporal lobe epilepsy (non-MTLE) group of 9 cases without temporal sclerosis, and MTLE group of 20 cases with temporal sclerosis. Non-MTLE group included 4 cases of focal cortical dysplasia, 2 cases of oligodendroglioma, 1 case of ganglioglioma, 1 case of focal neuronal hamartoma and 1 case venous angioma, with neuron loss less than 25% and low gliosis. In contrast, MTLE showed more than 50% neuron loss, high degree hyperplasia of astrocyte, and enhanced GFAP immunofluorescence. Transmission electron microscopy showed end-feet of astrocytes around capillaries were swelling and pressed vascular lumen and necrotic neurons. Conclusion: Our results that showed high degree hyperplasia of astrocyte and severe swelling of astrocyte in MTLE indicted the morphological and functional changes of astrocytes may play roles in the development of MTLE.
POS-TUE-001

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

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PURPOSE: Neurprecursors 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 neurprecursors remain unclear. The aim of the study was to compare the proliferation capacity and differentiation potential of skin-derived and brain-derived neurprecursors under in vitro culture conditions. METHODS: Periventricular regions were dissected from fresh post mortem canine brain and neurprecursors 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-ethyl-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 neurprecursors are morphologically similar under proliferation conditions and express neural stem cell markers nestin, NCAM and CD133. EDU assays revealed significant increases (p<0.05) in the proliferation rates of skin-derived (32.63%) compared to brain-derived (74.64%) neurprecursors 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 neurprecursors in terms of morphology and expression of neural stem cell markers. Skin-derived neurprecursors exhibit significantly decreased long term proliferation rates and have a decreased capacity for glial differentiation in vitro.

POS-TUE-002

GABA 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<sub>α</sub> receptor α<sub>1</sub>, α<sub>2</sub> and α<sub>3</sub> subunits in the piglet brain in the perinatal period. METHODS: GABA<sub>α</sub> receptor α<sub>1</sub>, α<sub>2</sub> and α<sub>3</sub> protein expression levels were assessed 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 0.3Mm sucrose for western blot analysis. RESULTS: GABA<sub>α</sub>1, α<sub>2</sub> and α<sub>3</sub> 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<sub>2</sub> and α<sub>3</sub> expression at P0 when compared with expression at P1. Conclusions: Knowledge of which GABA<sub>α</sub> receptor subunits α-isofoms 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 BARRSEL 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 birthdating study in 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, the CB was exhibited an abnormal distribution and were arranged in clusters of 4-5 neurons arranged in 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 as a novel interacting partner of Rnd2 which regulates the cell morphology and migration of embryonic cortical neurons.

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 Rho-protein signaling (Heng et al, Nature, 2008, Pacray et al, Neuron, 2011). However, the underlying molecular mechanisms remain poorly understood. Purpose: To characterise the signalling 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., Leamy 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 to 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 environment and that 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-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 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 (5K0 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 and co-regulatory functions of this gene in 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-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<sup>+</sup>) 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 neo<sup>+</sup> when compared to neo<sup>+</sup>. 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<sup>+</sup> and neo<sup>+</sup> mice injected with bromodeoxyuridine (brdu). Mice were analysed 2 hours and 7 days later. BrdU positive cells were co-labelled with Pdx6, 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 rostro-caudal axis (SVZ – RMS – OB). Results: This analysis revealed that after a 2 hour BrdU pulse there was a significantly higher number of Pdx6<sup>+</sup>/BrdU<sup>+</sup> (p<0.05) and DCX/ BrdU<sup>+</sup> (p<0.01) proliferating progenitors in the rostral migratory stream of the neo<sup>+</sup>. 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-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 cochlea were treated with the neurotrophin BDNF via transduction of mesenchymal cells with a BDNF gene construct to promote regeneration of the neurites. A further two weeks later the animals were euthanized with pentobarbitol 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 micro 5 gradient set. 3D gradient echo images were reconstructed with the following parameters: TR = 40, TE = 5.5, pulse = 35°, spatial resolution = 12.5 x 12.5 x 12.5 μm, n.s. = 4. Additional parallel experiments using conventional confocal laser scanning microscopy (Zeiss 710 NLO LSM) for β-tubulin immuno-fluorescence 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 blayer 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 radial fibre density when compared to the BDNF treated cochleae showing 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 periphery in the static cochlear turn. Conclusion: The high field MRI enabled determination of the full extent of the regenerated nerve fibre field within the reconstructed cochlea.
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 agangionated colon region. 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 agangionated colon when the colon is wrapped with a polymer scaffold seeded with NC cells.
**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 upregulated 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) vs older (E12) VM tissue in the presence or absence of E10 or E12 meningeal cells. Additionally, Parkinsonian 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 presence 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 in neurogenesis in the hippocampus is associated with improved memory of the hippocampus, a region important for memory and learning function in humans. **Methods:** and learning abilities, whereas increased levels of Cortisol and a neurogenesis in the hippocampus is associated with improved memory. The level of Adult Hippocampal Neurogenesis (AHN) can be regulated by and Docosahexaenoic acid (DHA) are known to have beneficial effects. **Results:** Cells were seeded on collagen coated plates to allow for optimal conditions. The number of neurospheres, increased fraction of EDU+ cells, as well as upregulated 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-016**

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

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**Purpose:** Teneurins (Ten-ns) 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 wildtypes (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. There is a significant 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 REINNERRATION 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 C57Bl/6J mice received 10 minutes of sham (n=9), 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 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 tasks 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 neuro grafting studies.

Methods: The human ES cell line '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. 1x10⁴ 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 heterogeneous 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 vivo 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 conclusions of graft integration with functional outcomes in animal models of brain damage.

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-operated. 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 in the EGL was consistent with 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 delay in EGL development. Proliferating granule cells exit the cell cycle, are equipped with a migratory giall scaffold but are unable to populate the IGL. This may be due to apoptosis or altered migratory cues; these possibilities are being assessed.
TRANSMEMBRANE 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 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 regions. Methods: Brains from embryos (E13, E16, E18) and neonates (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-labeled progenitors declined, but a greater association with VEGFR1 was seen in VEGFA and VEGFB expressing neurons. 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 neurons is consistent with VEGFR1 having an ongoing but different function in postnatal brain.

Purpose:

To investigate the cues involved in forming the ipsilateral retinocollicular projection, we used monoclonal enucleation to investigate the cues involved in forming the ipsilateral retinocollicular map. Methods: E18-E23 stage 13.5-14.5 days pregnant females were injected with electrophysiological recording in the SC when adult. Results: Following enucleation, the ipsilateral projection in WT mice mapped to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal 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 to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal 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 to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal 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 to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal 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 to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal 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 to the contralateral visual field. In KO mice, the ipsilateral projection was mapped to the contralateral visual field. Conclusion: The ipsilateral projection is established during development through mechanisms involving Ephrin-A5. The ipsilateral projection in KO mice has a normal...
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/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 neurotroph 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 purified promoting 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=5). 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 for 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, 114, 116, 118 and 119 d gestation. 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/mm²) 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 increased in the LPS brains (57 ± 20 cells/mm²) compared to control and LPS+hAECs (761 ± 13 cells/mm²)-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 become extensively characterized based on neuronal markers, the order 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 pulse-mated C57Bl6 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 GGRP immunohistochemistry, the mice were killed at 5-8 weeks of age by cervical dislocation, and the small intestine removed and processed for immunohistochemistry 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 vision 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 KO's (p<0.05, Mann-Whitney U-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 embryonic pre-migratory NCCs were electroporated with GFP plasmid at E1.5, then at E3.5 a fragment of forescut containing one GFP+ ENCC was isolated and combined with E4 ENCC wallfront. 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 logically 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 diversity at the level of individual cells.

POS-TUE-032

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

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Purpose: The Na1.2 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 more excitable than the 'adult'; moreover, a neonatal isoform was discovered that is only expressed in the inner fetal 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' Na1.2 is to reduce neuronal excitability in infant brains. To test this we have engineered a mouse line which only expresses 'adult' Na1.2 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' Na1.2, 1 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 wild-type and 'adult' Na1.2 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' Na1.2 neurons displayed increased susceptibility to PTZ-induced seizures compared to wild-type littermates (n=8, p<0.05) consistent with the idea that the 'neonatal' Na1.2 confers protection against hyperexcitability. Furthermore, homozygous 'adult' Na1.2 mice had an increased susceptibility to PTZ-induced seizures compared to wild-type littermates (n=5) displayed increased firing compared to the wild-type, consistent with hyperexcitability. Conclusion: 'Adult' Na1.2 neuron hyperexcitability and increased seizure susceptibility suggests that 'neonatal' Na1.2 may confer a degree of seizure protection during development.
POS-TUE-033
SEIZURE-RELATED GENE 6 ACTIVATES CALCIUM-DEPENDENT SIGNALING 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 in vivo.

METHODS: Changes in intracellular calcium levels ([Ca^{2+}]_i) were measured in cultured mouse cortical neurons, loaded with the calcium indicator dye Fluo-4-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 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 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 and β′ subunit modulation on the “excitatory neuron” α-subunit: Na1,2, and the “inhibitory interneuron” α-subunit: Na1,1.1. Methods: Automated patch clamp using the Nanion patchliner, was used analyse αβ subunit combinations transiently transfected into HEK293T cells. Results: Analysis of Na1,2 with the β′ 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). Na1,2 with β′ and β′′ subunit revealed a slower recovery from inactivation (p<0.01, N=19). In contrast, when Na1,1 α-subunit was co-expressed with β1,1 only parameter significantly modulated was recovery from inactivation, which was slower (p<0.001, N=13), when β′ and β′′ were co-expressed. However β-subunits did increase current density of Na1,1.1, which was not seen with Na1,2. Conclusion: We observed a differential effect of β-subunits on the “excitatory neuron” α-subunit Na1,2, and the “inhibitory interneuron” α-subunit Na1,1.1. If, in the context of disease, a β 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 deactivation during seizures. We hypothesise that the GABA2γ variant (GABA2γR43Q(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: Injections of HCN1 mRNAs into the hippocampus was assayed by qPCR in seizing and seizure-free mice. The effect on Ih 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 basal Ih current was reduced in injected DBA(R43Q) mice (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 a reduction in Ih 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 tested that HCN1 reduction may be a biomarker for hippocampal-based seizure co-morbidities.

POS-TUE-036
ALTED 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 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 days) 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 Fluor5F 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). 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 ANTI-EPILEPTIC 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 isofrom specific pharmacosensitivity using automated medium-throughput high content assay. Methods: cDNAs of human HCN1 and HCN2 were transfected 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 fitted with a Boltzmann equation to measure V. and slope before and after drug application.

Results: Lamotrigine (50μM) reversibly left-shifted the GCV curve of HCN1 (r=7.25±0.6 mV vs -75.8±2.7 mV, p<0.05, n=14) but was without effect on HCN2 channels (p=0.05, n=7). In contrast, gabapentin (100μM) a small (74.2±0.6 mV vs -79.5±0.7 mV, p<0.05, n=14) but was without effect on HCN1 and HCN2 channels as good antiepileptic drug targets. Subtype selectivity and differential modulation may be an important factor for future drug development.

Conclusion: Genetic and pharmacological reversible right-shift in the G-V curve of HCN2 channels was observed (p>0.05, n=7). In contrast, gabapentin (100μM) a small (-74.2±0.6 mV vs -79.5±0.7 mV, p<0.05, n=14) but was without effect on 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-039

STUDY OF THE DISRUPTION OF NERVE-CELL CONNECTIONS IN ALZHEIMER'S 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 neurotransmission. Synapses can 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 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 in sex-and age-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 recordings in retinal ganglion cells 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.
POSTERS

Tuesday

POSTER-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 CaMKII mutants (empty vector, wild-type, T253D, T286D, T253D/T286D, 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) followin single and double phosphomimetic mutation were examined (n=3). RESULTS & CONCLUSIONS: Using cell cycle and proliferative measures in a non-neural 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 phosphomimetic 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.

POSTER-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-hyperpolarization 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 soma 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. 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. Conclusions: 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.

POSTER-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, sucking, 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 12-15 week old C57Bl/6 mice (P7-10). Whole-cell recordings were made from visualized HMs and SMs in age-matched mice.

RESULTS & DISCUSSION: Single sweeps recorded from HMs demonstrated large amplitude (1.3 nA/s, maximum frequency 42.2 ± 4.0 vs. 26.9 ± 2.4 Hz, n = 17 and 10). HMs discharged at higher frequencies in response to square step currents compared to SMs (40%, p<0.0001). Subtractive cell-type separation in Grin2a null mice showed SMs maintained a high firing rate in response to depolarizing current, whereas HM firing rate was decreased. This suggests each population possess differing suites of ion channel currents that allow them to undertake their distinct motor functions.

POSTER-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-AMPAR 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=10 pairs) and confocal-immunofluorescence (n=3 pairs). Quantitative post-embedding immunogold-electron microscopy was used to compare GRIP levels in stargazer and control cerebellum and thalamus.

Results: GRIP-P1 and GRIP-P2 expression were decreased (40%, p<0.0001) and (70%, p<0.01) in stargazer cerebellum and thalamus, respectively. PICK1 expression was unchanged at synapses (n=4 pairs, 280 synapses). GRIP was expressed at both excitatory and inhibitory synapses in thalamus and cerebellum (Grin1 and Grin2a mRNA expression were unchanged in stargazer compared to control). PICK1 was expressed at thalamic synapses at excitatory synapses (Grin1 and Grin2a mRNA expression were decreased in stargazers).

Conclusions: In stargazer cerebellum and thalamus, GRIP-P1 and GRIP-P2 expression is decreased, whereas PICK1 expression is unchanged at excitatory synapses. This suggests that the Stargazer mutation impacts GRIP-P1 and GRIP-P2 expression, but not PICK1 expression.
POSTERS

**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 (5th, 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 5th, 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 neurotransmitter receptor TrkB (30.6726.7%, n=5) and VAMP2 (27.0849%, 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 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; Nav1.2, 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 processes. The Nav1.2 channel may relate to a 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**

INCORPORATION OF POLO-LIKE KINASES INTO THE REGULATORY MECHANISM OF \( \alpha \)-SYNUCLEIN 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 \( \alpha \)-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 intraperitonially 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 bloting of tyrosine hydroxylase (TH), \( \alpha \)-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 \( \alpha \)-synuclein in the striatum, 2) a dramatic increase in the levels of phosphorylated \( \alpha \)-synuclein in both nigra and striatum, and 3) a correlation between increased \( \alpha \)-synuclein phosphorylation and increased levels of PLK-2 and PLK-3 in the both the nigra and striatum. The levels of all proteins trended to increase and phosphorylate \( \alpha \)-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 interferences between concurrently run stimulus presentation and data collection functions. Results: Our system runs a specialized True3DI/AOCT 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). We studied the distribution of these cells in various visual cortical areas (V1, V2, V4A, 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 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/day, 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 Ctip2/RIBEYE, Vgat, VMAT2, and PMC1A1, all of which are abnormally expressed in retinal neurons. Conclusion: These data suggest that the abundances of proteins associated with ribbon synapses are susceptible in diabetic retinopathy. Further work is necessary to determine the mechanism causing these protein alterations.
POSTERS

Tuesday

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 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 38.6% (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 15% (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 antagonists 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/RFP, 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 (C1q, 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

da9-NICOTINIC ACETYLCOLINE 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 a-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 a9 and a10 subunits. However, antagonism of the a9-a10 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 a9a10 nACHRs can contribute to chronic pain, several sciatic nerve injury models were tested in a9a10-nACHR-knockout (KO) and wild-type (WT) mice. Results: KO (n=6) mice develop mechanical allodynia (von Frey and incapacitation tests) that is indistinguishable from WT’s (n=6), which persists for at least 3 weeks. Mechanical hyperalgesia (paw pressure test) also develops in the KO (n=6) mice. KO mice show no difference in behavioral responses to mechanical allodynia or hyperalgesia compared with WT. Vc1.1 significantly reduced mechanical allodynia (94% of pre-surgical response; p<0.05, n=21). Conclusion: Our results show that activation of a9-a10 nACHRs contributes to chronic pain in these models. a9-a10 nACHRs inhibit N-type Ca2+ channels and also modulate GABAB receptor activity, and Vc1.1 may act through both mechanisms.

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 potency and selectivity 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-conotoxin CVID and CVIE 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%. However, no significant difference between maximal inhibition by CVID and CVIE. In DRG neurons from adult rats, complete recovery was seen following washout of CVID in all cells tested (n=9). Recovery from CVIE was more variable, with no recovery in 4 cells, partial recovery in 1 and complete recovery in 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 CONTRIBUTION 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 allosthenia 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. Thereafter, 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 block). 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-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, rdf-FITL, 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, rdf-FITL-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 P105 rdf-FITL-Thy1 and control mice were prepared as wholemounts and processed using immunohistochemistry. Results: Ganglion cells (cells=1065, n=28) were classified based on 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-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 investigate the sensitivity of neurochemically identified cell populations to kainate. Within the inner retina, OFF cone bipolar cells were the most kainate sensitive inner retinal neuron population with a K_{EC} 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 most kainate sensitive than 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-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. Two-way ANOVA 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 600μm, 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 hemispheres 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%, difference +2±1%, p<0.01). This difference saturation changed at -2.4% for each 10μm increase in vessel width.

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 (konioacellular, 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 (VI) [1]. Our purpose here is to find the source of this variability, specifically its time-motion of lower frequency EEG. **Methods:** Extracellular spike activity of LGN neurons (n=107) and local field potential from primary visual cortex (VI) were recorded in sufenhanti-anaesthetised marmosets (Callithrix jacchus, n=12). The visual stimulus was a uniform grey field ~15 degrees square at close to 50 Cd / m². Granger causality analysis was performed on LGN neuron maintained discharge rate and VI 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 means±SD. **Results:** As reported [1] KC neurons showed high variability in maintained discharge rate variability (36.83±4.67, n=37) compared to Parvocellular (9.13±0.84, n=45) and Magnocellular (12.5±2.1, 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 (0.3±0.2). **Conclusion:** These results indicate that decreases in delta frequency oscillation strength in the primary visual cortex cause increases 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) 6, 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 BA6 complex, which produced a clear topographic segregation of claustrum connections: labelled cells projecting to the medial subdivision (area 6b) 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 numerous centrally. 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 a potential option for the treatment 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 brainstem 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.
POSTERS

Tuesday

POSTER: Tuesday

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 current (Ih). At the resting membrane potential, 3.5% of the maximum Ih conductance was available. Inhibition of Ih hyperpolarized the membrane by 6.m and defracted spontaneous and a subsequent inhibitory component. Inhibition of Ih reduced the frequency of these biphasic events by 65% and increased the decay time constants of the inhibitory component. Conclusion: Ih 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.

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 intratheacial. 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 CVIE and CVID 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 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.

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 the rostral-caudal direction along the large bowel; and identify their specific targets. Methods: Lesions were made to the lumbar splanchic and rostral-caudal direction along the large bowel; and identify their specific targets. Methods: Lesions were made to the lumbar splanchic and rostral-caudal direction along the large bowel; and identify their specific targets. Results: After 7 days, anterogradely labeled axons were found to ramify up to 18mm oral and 9mm anal along the colorectum and 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. Conclusions: while retaining complete neural continuity with the large intestine in vitro. Conclusion: Our results demonstrate that spinal afferent nerves innervate in the rostral-caudal direction along the large bowel and extend up to at least 18mm oral and 9mm anal along the colorectum.

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-HT1A 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-HT1A receptor. Methods: We used two serotonergic drugs in healthy participants (n=12): citalopram to increase global levels of serotonin, and buspirone to activate 5-HT1A 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 effects of the drugs on rivalry switch rate. Individual differences in reactions to the drugs may also play a role.
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 distractors. PURPOSE: In this study, we assessed the effectiveness of location and feature cues in the presence and absence of a highly salient distractor. 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 distance 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 distractor was also presented at one of the non-target locations. This distractor had features that were either similar or dissimilar to the target.

RESULTS: It was found that the presence and absence of a salient distractor 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 distractor influenced performance (Repeated Measures ANOVA, P<0.05). Distractors 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 distractor.

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 different orientation 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 stimuli of membrane potential responses. The source of such invariance seen in striate cell membrane potential responses. The source of such invariance seen in striate cell membrane potential responses 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.

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<sup>min</sup>, rd8) on the background strain. Our aim was to characterise the retinal effects of lack of Ccl2 and Cx3cr1 (Ccl2<sup>-/-</sup> Cx3cr1<sup>CNGt/DNg</sup>, 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<sup>CNGt/DNg</sup>- mice but not Ccl2<sup>-/-</sup>- founder lines. This correlated with absent 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.

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

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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. Methods: Antibodies against glutamic acid decarboxylase 65 (GAD-6) to label GABAergic amacrine cells, glycine transporter 1 (GlyT1) to label glycinegic 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<sup>2</sup> (measured using DIC optics) is close to the sum of GABAergic (17707 cells/mm<sup>2</sup>) and glycineergic (19776 cells/mm<sup>2</sup>) amacrine cells. In the ganglion cell layer (GCL) no glycinegic cells are present and the density of GABAergic (GAD-6 positive) amacrine cells is 3073 cells/mm<sup>2</sup>. 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.

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

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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 different orientation 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 stimulus for membrane potential responses. The source of such invariance seen in striate cell membrane potential responses 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.
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 after 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 post-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.

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 imaging and 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 reprofuting 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.

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 lesining 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 the visual 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. The perimodal 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 mice 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.

**POSE-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 ribonucleoprotein (hnRNP)s. 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 relocated 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 hnRNPK 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 pathophysiologies associated with mutated TDP-43 in ALS, and FTD patients.

**POSE-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. Lesions to the RST selectively abolish the arpeggio movement. The results support the view that the arpeggio movement is under the control of the RST. RN lesions, however, create 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)

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 (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 TDP43-43 mice at 60 days, as well as in NSC-34 cells stably expressing mutant TDP-43. Confocal microscopy and cell fractionation revealed that active phosphorylated 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=4) 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 are interested in examining the neuronal organisation of the motor columns in the rat forelimb (Tosolini and Morris, 2012). Using a combination of intramuscular injections of viral vectors and the ensuing retrograde transport of the therapeutic gene into targeted motor neurons, 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 plate (MEP) sites 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 by electron microscopy. 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: The motor end plate map and the motor column map constitute a valuable guide for the selection of appropriate muscles 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 PRECERCEREBELLAR NUCLEI IN THE C57BL MOUSE - NEW INSIGHTS FROM TRACING AND GENE EXPRESSION
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PURPOSE To make a comprehensive map of the precerbellar (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 Wnt, Math1, and Hoxa3 cre lineages to characterize the mossy and climbing fibre-issuing PreCb groups of the hindbrain. RESULT In addition to the classical five precerbellar 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 PreCb 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 parafascicularis, 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 ‘rhythmogenic 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 expiratory activity (RELAY) directly 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 transsections 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 C4. Conclusions: Ascending synaptic inputs arising from a very caudal medullary and high cervical spinal cord area (A1 cell group, retroambigous?) are essential for maintenance of mammalian respiratory rhythm generation.

POS-TUE-088

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 (RVL/M), sense the reduction in glycaemia and increase the adrenergic 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 glycaemia, 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 RVL/M (N=6) neither changed ASNA (101±1% vs 84±14%) nor blood glucose (6.4±0.1 vs 6.9±0.3 mmol/ml). On the other hand, subsequent intravenous infusion of 2DG (300 mg/kg) increased ASNA (101±1% vs 183±28%) 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 RVL/M, are glucose sensitive and their excitation produce adrenaline release in response to hypoglycaemia. Supported by the NHMRC.

POSTERS

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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 ‘rhythmogenic 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 expiratory activity (RELAY) directly 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 transsections 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 C4. Conclusions: Ascending synaptic inputs arising from a very caudal medullary and high cervical spinal cord area (A1 cell group, retroambigous?) are essential for maintenance of mammalian respiratory rhythm generation.

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 nerve endings during nicotinic blockade enhanced recovery of peristalsis during nicotinic blockade. Method: Segments of proximal ileum (5-7cm) were taken from guinea pigs (39±12g) of either sex and cannulated and pressure recordings were made (anal end). Video recordings were made of intestinal movements and drugs were added directly 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) ileum (500 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±1.3mmH2O versus HEX: 40.8±2.2mmH2O). Twelve showed no recovery but 4 of 22 partially recovered with 2.5 out of 6 attempts successful (PT control: 32.5±2.5mmH2O versus HEX: 43.5±4.8mmH2O). Neither the nicotinic antagonist mecamylamine (3μM; n=6), nor a combination of nicotinic and muscarinic M1 antagonist (VU-0255035, 150μM; 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

ARCULATE NEUROPEPTIDE Y CONTROLS ENERGY EXPENDITURE VIA A PARAVENTRICULAR NUCLEUS RELAY

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Neuropeptide 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 with no 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 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 expenditure via influencing BAT thermogenesis.
POS-TUE-089

DOWN-REGULATION OF AQUPORIN 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 idiosyncratic nature. Aquaporin (AQPs) 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 colon 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 AQPs1-11. 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 observed in STC mucosa. Immunoreactivity for AQP1 and AQP3 was mainly seen 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 intermedioleseral 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’, which is unclear. 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 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 splenic and plasma TNFα levels remained low. In LPS treated animals after sham surgery, plasma TNFα rose to 172±25pg/ml; and spleen TNFα to 192±23±4pg/100mg. The corresponding levels in LPS-treated animals after splanchnic nerve section were 253±6117pg/ml and 298±70pg/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 cultured 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 neurones 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 viral infections.
POSTS

POSTER 158

POST-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 transynaptic 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 H129 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 replication 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.

POST-TUE-094

PHARMACOLOGICAL ANALYSIS OF SYMPATHECTOMICALLY-MEDIATED CONSTRICITION 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 α1 and α2 adrenoceptors (ARs), acting synergistically [1] whereas, in vivo, α2 but not α1 AR blockers cause vasodilation, increasing tail skin temperature [2]. **Methods:** Male C57Bl mice (4-8 months) were killed with CO2 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 (α1-AR antagonist), 100 nM rauwolscine (α2-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 (~60%) 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] Yeh Y, 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.

POST-TUE-095

CHEMOTHERAPY INDUCED DIARRHOEA: TARGETING SECRETOMOTOR NEURONS

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**Purpose:** Common side-effects of anti-cancer chemotherapy include nausea, vomiting, constipation, diarrhea. 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) 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.

POST-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. **Conclusion:**: Pro-emetic interventions observed in our study were quite robust and sensitive to antiemetic 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 for the extent of the lesion. Cap treatment resulted in a diminution of the acute and chronic effects of AGB on AGB of NTS neurons and an amelioration of the AGB-induced reduction in food intake, body weight gain, fat mass and feed efficiency (p<0.05). Associated with this reduction in body weight and fat mass in obese rats, there was 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<0.05). 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 days 14 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 (RLVM) NEURONS THAT PROJECT TO THE CONTRALATERAL BRAINSTEM

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Purpose: Previous experiments indicate that the contralateral brainstem is a target of ouabain derived to RLVM sympathetic premotor neurons, but the identity of neurons responsible are unknown. This study examines distribution, phenotype, behaviour and post-synaptic targets of contralaterally projecting RLVM neurons. Method1: Contralaterally projecting neurons were identified by a combined retrograde-antegrade tracing strategy. Latex beads and dextran were co-injected into the RVLM of contralaterally projecting RVLM neurons.

Results: Many RVLM neurons were antidromically activated. Neurons projecting to the contralateral brainstem were widespread, most are apparently 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.

Results2: In part, by orexin acting at OX1 and OX2 receptors, contralaterally projecting neurons were identified apparent synaptic contacts on sympathetic premotor neurons. Conclusions: While RVLM neurons with projections to the contralateral brainstem are widespread, most are apparent 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.

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-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 (OX1-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 activated in response to stimulation of the LH region. Results: 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 induction of peri-stimulus time histograms. LC neurons responded to LH stimulation with an on latency of 6.5±0.4 ms and a peak latency of 15.2±0.8 ms. Administration of the OX1-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 OX1-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.
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 - neureminidase (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 enzyme injection. RESULTS: NEU (n=8) compared to vehicle (n=7) evoked significant increases in BP (22 ± 4 ± 5.3 ± 11% mmHg), HR (41 ± 10 ± 15 bpm) and SNA (32 ± 12 ± 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. Microinjection into other areas is underway (RVLM/CVML).

NGR1 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 SOC3 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, SOC3 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.

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 ± 3 mmHg; vehicle 0.37 ± 1.9 mmHg) and prelimbic cortex (PrL) (32 ± 0.6 mmHg; vehicle 0.68 ± 1.3 mmHg). 5-HT into the mPFC (n=4) also decreased AP with the greatest falls again seen in Cg1 (36.85 ± 4.17 mmHg) and PrL (16.84 ± 4.9 mmHg). 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.

SEX DIFFERENCE IN THE EXPRESSION OF OESTROGEN RECEPTOR ALPHA WITHIN NORADRENERGIC 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.

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 ± 3 mmHg; vehicle 0.37 ± 1.9 mmHg) and prelimbic cortex (PrL) (32 ± 0.6 mmHg; vehicle 0.68 ± 1.3 mmHg). 5-HT into the mPFC (n=4) also decreased AP with the greatest falls again seen in Cg1 (36.85 ± 4.17 mmHg) and PrL (16.84 ± 4.9 mmHg). 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-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 incapability of suppressing food intake compared to control mice. Importantly, TS treatment reinstated 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-109
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 lentil-R3/IF-GFP viral construct which encodes transduced neurons to secrete the selective RXFP3 agonist ‘R3/IS’ and GFP were bilaterally injected into the PVN, and mice were culled 2 weeks later and compared to lentil-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 lentil-R3/IS-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 suggest 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 (KCl SO4-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-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 revealed that BDNF stimulates Fyn autophosphorylation (n=3) which appears to be a Fyn dependent effect, as shRNA-mediated knockdown of TrkB in oligodendrocytes prevented the Fyn autophosphorylation. 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 reduces 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). Coronal 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). The aim of this study is to assess transfection efficiency and specificity of the OX42-immunogene for microglia and whether this vehicle stimulates an immune response in microglia.

Methods: 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.

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)1-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 cytoxic 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 macromolecules.

Methods: Neocortical microglia isolated from mixed cultures were placed in Krebs HEPES buffer and treated with substances to induce macrophage formation via P2X4 receptors (50μM, 100μM ATP). P2X7 receptors (100μM BzATP) or both (1mM ATP) in the presence of minocycline. The drug minocycline inhibits microglial activation in vivo, but its mechanism of action is not clear. We therefore investigated whether minocycline may block an early event in microglial activation, namely formation of membrane pores (macropores) that allow influx and efflux of macromolecules.

Results: Activation using 100μM ATP to target P2X4 receptors caused macrophage formation in 92% of microglia tested (n=52), whereas activation using 1mM ATP caused macrophage formation in only 24% of cells (n=27). Similarly the P2X7 agonist BzATP caused 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.
**POSTS-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. 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 mice) express DTR in SCs, rendering these cells sensitive to DT-mediated apoptosis.

**Results:**
In the sciatic nerve of MBP-DTR 25+DT mice but not control wild-type one intraperitoneal (i.p.) injection of 10ng/g DT, we observed apoptosis 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 CC 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.

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**POSTS-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.

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**POSTS-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. Here we aim to determine the relative contribution of NPCs and OPCs to oligodendrogenesis following cuprizone-induced demyelination. Methods: Adult NestinCreER^22, Rosa26-lox-STOP-lox-YFP mice were administrated 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=8) demonstrated that approximately 60% of YFP+ NPCs commit to an oligodendroglial fate. There was robust recruitment of NPC-derived oligodendroglial cells, with an 14-fold increase in YFP+Sox10+ cells compared to unmatched challenged mice (n=3). Most of these cells were mature oligodendrocytes (YFP+CC1+) whose 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.

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**POSTS-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 administration 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 stereotactic 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.
**POSTERS**

**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 arylnactelamides agonists and the novel neoclerodane diterpene Salvinarin A (Sal A) attenuates 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 lever 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 assessed and 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 naive or cocaine self-administering rats (n=7). However, MOM Sal B significantly suppressed operant lever press responding for sucrose reinforcers (P < 0.01; n=5-7) suggesting a non-selective effect on reward. **Conclusions:** This study confirms that 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 neurexins are cell adhesion molecules important for formation, modulation and activity-dependent plasticity 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 molecules experimentally. **Methods:** We are employing behavioural assays to assess the function of neurexin and neurexins, 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 *neurexin2* (n=22,37) leads to more sleep bouts compared to wild-type (n=131, p<0.05); however this change is also seen in apparent Mecamylamine (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 neurexin (of four) may be more subtle, possibly due to compensatory genetic mechanisms; follow up experiments using knockdown 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**

**REGIONAL 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 HD are unclear; the specific mechanisms underlying the disease remain unknown, but may involve pathological changes from other areas of the brain. This study investigated 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 cerebellum 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 gial-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.
POSTERS  Tuesday

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<tr>
<th>POS-TUE-121</th>
<th>DEFINING THE REGULATION OF AMYLOID PRECURSOR PROTEIN N-TERMINAL FRAGMENT GENERATION</th>
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<tr>
<td>Liu L., Ciccottosto G.D. and Cappai R.</td>
<td>Department of Pathology and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, Australia.</td>
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<td><strong>Background:</strong></td>
<td>The Amyloid Precursor Protein (APP) of Alzheimer's 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, mempin-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. <strong>Methods:</strong> Neuronal KCl depolarization: SH-SYSY 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-SYSY cells or mouse cortical neurons were depleted of cholesterol by replacing the growth media with fresh media containing 5 mM methyl-β-cyclohexatin. After 10 and 20 minutes of treatment, the analysis was collected. 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: Neuronal KCl depolarization treatment resulted in an increase in APP-NTFs production at the 5 and 120 min time points. An increase in secreted APP ectodomain was also measured. <strong>Conclusions:</strong> 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 mempin-beta activity is being investigated.</td>
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<th>POS-TUE-122</th>
<th>ELECTROPHYSIOLOGY OF NUCLEUS INCERTUS NEURONS: HETEROGENEOUS RESPONSES TO CRF AND COHERENCE WITH HIPPOCAMPAL THETA RHYTHM</th>
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<td>Ma S.1,2, Blasiak A.3, Oluocha-Bordonau F.E.1, Verberne A.J.M.2 and Gundlach A.L.1</td>
<td>1Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australia. 2Dept of Medicine, Austin Health, University of Melbourne, Heidelberg, Australia. 3Dept of Neurophysiology and Chronobiology, Jagiellonian University, Krakow, Poland. 4Dept of Anatomy and Human Embryology, Universidade de Valencia, Valencia, Spain.</td>
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<td><strong>Purpose:</strong> Relxin-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-tracing, in vivo extracellular unit recordings with juxtaacellular 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. Conclusions: 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.</td>
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<th>POS-TUE-123</th>
<th>DYSFUNCTION IN SOCIAL COGNITION BUT SPARING OF SHORT-TERM SPATIAL MEMORY IN A MOUSE MODEL OF METHAMPHETAMINE-INDUCED PSYCHOSIS: CONTRASTING ROLES OF BDNF</th>
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<td>Manning E.E.1,2 and van den Buuse M.1,3</td>
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<td><strong>Purpose:</strong> 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. <strong>Methods:</strong> 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/counts in the social interaction task were analyzed with Cleversys TopScan software. Group differences were analyzed with repeated-measures ANOVA and paired t-test. <strong>Results:</strong> 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 HE Ts (p=0.02). In contrast, during the novelty preference phase when a new stranger mouse was introduced, METH-treated BDNF HE Ts showed selective disruption of the social preference over WT mice. METH treatment did not affect the preference for the novel mouse (paired t-test). <strong>Conclusions:</strong> 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.</td>
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<th>POS-TUE-124</th>
<th>EFFECT OF WESTERN DIET CONSUMPTION ON SPATIAL ALTERNATION AND BRAIN SEROTONIN MEASURES IN THE RAT</th>
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<td>Nguyen J.C.D.1, Bongiorno D.1, Killcross A.S.2 and Jenkins T.A.1</td>
<td>1RMIT University. 2University of New South Wales.</td>
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<td><strong>Purpose:</strong> 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, epidymal fat, basal locomotor activity and spontaneous alternation behaviour in the Y-maze in a western diet rat model of consumption. <strong>Methods:</strong> 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. <strong>Results:</strong> While the consumption of the western diet did not significantly increase final body weight (39±2 g vs. 37±2g, control and western diet fed rats respectively, p=0.099) it did produce a significant increase in epidymal 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 was observed in the striatum (p=0.038). <strong>Conclusion:</strong> The consumption of a western diet in male Long-Evans rats for 12 weeks resulted in the increase of epidymal fat weight with an associated increased expression of 5HT-2C receptors in the striatum. These observed increases in epidymal fat and 5HT-2C receptors are not affected by body weight.</td>
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A META-ANALYSIS OF BRAIN AREAS ALTERED IN DEPRESSED SUBJECTS

Palmer S.M.1 and Carey L.M.2,3
1Stroke, Florey Institute of Neuroscience and Mental Health, Australia.
2Occupational Therapy, LaTrobe University, Australia.

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.

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

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Cognitive and Exercise Neuroscience Unit, School of Psychology, Deakin University, Melbourne.

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 investigated 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 (DLPFC), 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: 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.

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 C57Bl6 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 withheld 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. Following 8 days of extinction 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.

A ROLE FOR THE MGlu5 RECEPTOR IN EXTINCTION OF COCAINE-SEEKING BEHAVIOUR

Florey Department of Neuroscience, University of Melbourne.

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 operand 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) or saline. Results: Following 8 days of extinction, 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 > 0.05). Responding was reliably reinstated by the cocaine prime (p < 0.05). 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 saline (p < 0.05), and 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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SCHOOL OF PSYCHOLOGY, UNSW.

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 morpholino to study the specific role of the orexin neuropeptides in context-induced reinstatement of alcholic 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 morpholino 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 morpholino. 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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1School of Biomedical Sciences and Pharmacy. 2Centre for Translational Neuroscience and Mental Health Research, HMRI. 3University of Newcastle, Australia.

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 DLS. 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

Ratnayake U., Dickinson H. and Walker D.W.
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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 day 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=12-18) 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 (wMe) or extreme (wMelPop) 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 = 5/condition). qPCR was used to estimate changes in gene expression of four genes within the dopaminergic biosynthetic pathway (dop-1, ppo-ll, 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 wMelPop infected flies. Changes to 24 dopamine biosynthetic pathway genes was observed, the greatest impact was associated with the wMelPop 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 antagonist (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 reproducibly identified proteins in treated rats, and 964 in control rats, indicative of oxidative stress, for example nitric oxide synthase, were also found. Differentially expressed proteins indicative of oxidative stress, for example nitric oxide synthase, were also found. Conclusion: The hippocampal proteome induced 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 ethanol 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 ethanol 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. Our lab, RHODES, B. J. S., BEST, K., BELKNAP, J. K., FINN, D. A. & CRABBIE, 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 developmental 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. 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 C57Bl/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-agonist 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=13,16, 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 dysregulated 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 Bonferroni 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 85 dBA 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-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, 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 (1mg/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-B mRNA expression was significantly upregulated in the IF while GAD-2, GAD-1 and GAT-3 mRNA expression were significantly upregulated in the PRL. Ionotropic GABA-A receptor subunits α1, α3, δ5 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-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% O2). Controls were subjected to 10 min of O2 (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 increased freezing behaviour compared to no-anaesthesia controls, indicating a memory impairment (25.4±5.4% vs 66.2±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 and decreased in anti-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 hippocamp (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-HT2A RECEPTOR BINDING IN THE RAT BRAIN
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Olanzapine is widely used in treating multiple domains of schizophrenia symptoms through its binding profiles to various neurotransmitter receptors including 5-HT2A receptors (5-HT2A, R). Our previous studies have shown that 2 weeks co-treatment of betahistine (a H1R agonist and H R antagonist) could reduce obesity induced by olanzapine. This study aimed to investigate whether long term co-treatment of olanzapine and betahistine affects 5-HT2A, 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-HT2A, R were measured using [³H]ketanserin. Results: Compared to the controls, olanzapine significantly decreased 5-HT2A, 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-HT2A, R bindings only in SN (p<0.001). Conclusion: Co-treatment of olanzapine and betahistine had similar effects as sole olanzapine treatment on 5-HT2A, 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-HT2A 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, CuII(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 CuII(atsm) elicits these effects. As oxidative damage and astrocyte activation were attenuated by CuII(atsm), potential stimulation of the neuroprotective antioxidant systems of astrocytes was investigated in vitro. Methods: Primary astrocytes cultured from mouse brains were treated with CuII(atsm) for 24h (n=3). Activation of signalling kinases was determined by Western blot to confirm the biological activity of CuII(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 CuII(atsm)-treated astrocytes to determine the influence of astrocyte-derived glutathione on neuronal glutathione content. Results: CuII(atsm) induced phosphorylation of kinases including Akt and ERK, and activation of Nrf2. Accordingly, CuII(atsm) treatment upregulated HO-1 and GCL, the latter enhancing glutathione content and elevating export of glutathione from astrocytes. Media from CuII(atsm)-treated astrocytes increased neuronal glutathione content. Conclusions: These results demonstrate that CuII(atsm) activates the transcription factor Nrf2 and upregulates astrocyte antioxidants. This action may contribute to the neuroprotective and disease-attenuated activity of CuII(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 vitreous 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 intraocular pressure (IOP) elevation in 2 and 14 month old rats. Methods: (ERG), ocular blood flow (OBF) and vitreal oxygen tension (pO2) during the same level of function and show poor recovery from stress, despite higher blood flow recovery. This was followed by two hours of recovery. Throughout the protocol photopic ERGs (background: 15cd/m2 stimulus: 2.03 log cd·ms -2) OBF and pO2 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 vitreous 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 IN THE AMYGDALA KINDLING RAT MODEL OF TEMPORAL LOBE EPILEPSY
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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-2A catalytic 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 amygdala kindling rats had significantly greater the PP-2A activities and PR55 expression, and increased 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 that the levels of phosphorylated-TDP-43—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 MT1 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 p75NTR-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 pathway in cell lines, 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 analyzed 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 Seize-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 (CCl) 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 denticulate spines per neuron in Sez-6−/− mice with CCl, 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 assayed using NanoSIMS. Results: Calcium ions were observed as microdomains which were divided into two categories: those associated with phosphorus (P), and those without. Microdomains are intracellular aggregates of calcium ions that may be located at the plasma membrane, in mitochondria and/or endoplasmic reticulum. In normal uninjured tissue, there was a greater proportion of non-P associated microdomains (P associated = 0.12 ± 0.05; non-P associated = 0.88 ± 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 [H]MK-801 and [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 mGluR2 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 males, this was not reflected in NMDAR 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 glycine 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 analgesic drugs that selectively target disease-affected GlyRs. Pain sensitization confers a unique conformational change in the α3 GlyR, important for two reasons. First, they provide the first direct evidence 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, this might also be applicable to any ligand-gated ion channel family member, and thus suggest new loci for phosphorylation producing extracellular conformational changes important for two reasons. First, they provide the first direct evidence 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, this might also be applicable to any ligand-gated ion channel family member, and thus suggest new loci for phosphorylation producing extracellular conformational 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 both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. Success: These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs.
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 (Tyr3, 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 Tyr3 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 Tyr3 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 Tyr3 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. Conclusion: 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 Marramou’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 estradiol (HE) 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 prominence 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: In-vitro study investigated type-I IFNs in post mortem human brain tissue, and in-vivo using human BE(2)M17 neuroblastoma (M17) cells treated with the PD associated neurotoxin, rotenone. Results: Using qPCR, we observed increasing expression of IFNA (30-fold, n=5) and IFNB (5-fold, n=5) was identified in prefrontal cortex confirmed a 3- and 4-fold up-regulation of IFNA and IFNB in PD patients, compared to age matched controls (n=10, *p<0.05). In-vitro studies confirmed rotenone (10μM-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 IFNB (50-fold, n=5) and IFNG (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, TNFα and IL-6. Significantly, stabilly expressing M17 Interferon Receptor-1 (IFNAR1) knockdown cells, showed significantly reduced levels of IFNA, IFNB, IL-1β and TNFα 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 500μM 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. Methods: The middle cerebral artery was occluded using intraluminal filament for 1 h followed by 6, 24 or 72 h recovery in Nox2-/- and Nox2+/+ mice. ROS were detected in-situ using dihydoroethidium (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 the 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

AUTOPIHAGY 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-methamphetamine, MDMA) via actions on biogenic amine receptors 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 mouse) 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 [HIS]-uptake (n=3) indicated reduction of programmed cell death in a concentration-dependent manner by oxidative stress (hydrogen peroxide; IC50, 100 µM), autophagic stressor (rapamycin; IC50, 15 µM) and MDMA (100 - 1000 µM). 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 (LC3) showed 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, especially 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-/-; Trf2mut) and double mutant Hfe-/-xTrf2mut mice, all on AKR background (ages 12-52 weeks; normal chow or short-term high iron diet). We assessed brain iron by indirectly 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 4x higher in Hfe-x Trf2mut double mutant mice (p<0.025, n>3/group). Various important transcripts showed changes in two or more models, including Fos and Camk2a. All models showed transcript changes relating to lipofuscin diseases but only Hfe-x Trf2mut mice showed strong evidence for an iron-activated inflammatory response. 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 ferrooxidase. 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 remains unclear. 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 STAT3 phosphorylation (a downstream mediator of type-1 IFN signalling) in neurons (FOX3 positive) of the frontal cortex of APP/PS1 brains (n=5). This staining surrounded amyloid plaques, accompanied by elevated astroglisia (GFAP). We previously demonstrated that IFNAR1-/- neurons are protected following Aβ1-42 insult through decreased IFN production and increased astroglial response. Here we further characterised the specific cellular responses involved in AD, primary cultured glia were treated with Aβ1-42. Wildtype 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, IFNα, IFNβ-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 JaZIP-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 encephalomyelitis (EAE). Methods: EAE of LEA lead to the upregulation of C/EBPδ mRNA expression and is an important regulator of Th17:Treg balance. 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 is 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 PHOTOSHADOWY 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 (A beta) polypeptide extracellular deposition and formation of intracellular neurofibrillary tangles (NFTs), along with dystrophic neurites. Evidence suggests that oligomeric A beta can induce neuritic dystrophy. The purpose of this study is to investigate how A beta 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 A beta (0.5, 1.0 and 10mM for 24h), to define which A beta-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 A beta-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 periaqueuductal 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 increased (~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 this subset of rats.

POS-TUE-167
POTENT ANTI-INFLAMMATORY EFFECTS OF ANDROGRAPHOLID AND ITS MAJOR METABOLITE, ANDROGRAPHOLID 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 IC50 values of 12.4±0.6µM and 14.2±0.3µM, respectively. Both compounds were much more potent than the NSAIDs aspirin and ibuprofen or paracetamol (IC50 values > 1 mM). The LC50 concentrations for andrographolide and andrographolide sulfonate were determined to be 1.2±0.2 µM and 4.8±1.1 µM, respectively. Conclusion: The increased 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 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 µg/kg/day BPA) everyday for the entire course of their gestation (19 days). 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 treated 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.
POSTERS

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 arrays (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.

POSTERS

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 role of 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 damage 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, midline 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 neurite outgrowth 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.
POSTERS

**POStue-173**

**IMMUNOGLOBULINS IN FRONTAL TEMPORAL 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 (IgG) proteins in FTLD tissue. Differences were observed between the tau-positive and tau-negative subtypes of FTLD which suggest different underlyingimmune processes. **Methods** Following institutional approvals, tissue was obtained from the Sydney Brain Bank: normal controls (n=1), FTLD (n=15) 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-negative 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.

**POStue-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-8 and TNFα mRNA were assessed. **Methods**: Adult male Sprague-Dawley rats (n=20) were anesthetised 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 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 TNFα 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 TNFα (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.

**POStue-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 therapies act through DRD2-like dopamine receptors after conversion to dihydrotestosterone (DHT) and through estrogen receptor after aromatisation to 17β-estradiol (E2) to control transcriptional effectiveness. Testosterone acts through androgen receptor directly or via androgenic mechanisms. Two families of dopamine receptors exist: excitatory DRD1-like (DRD1, DRD5) and inhibitory DRD2-like (DRD2, DRD3, DRD4). Current antipsychotic therapies act through DRD2-like dopamine receptors 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 adolescent males 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 drug vehicle treatment 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 reduced DRD1 and 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.

**POStue-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 protective effect. 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 life of a large fraction of the population. Objective: We aimed to assess the effects of immunisation with non-encephalitogenic myelin-derived APLs on 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 proliferation 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, CFA only or CFA 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, CFA or CFA only-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 ANTISACCADIC 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). Methods: 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 saccadic 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 were then repeated testing the young mTBI group to compare 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 speed 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 symptomatic HD and 18 healthy controls (n=4, days 10 and 30 post-CCI) and nine networks. Voxel-wise synchronization of networks of interest were compared between groups using dual-regression and voxel-wise analysis. 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 increased synchrony in symp-HD, compared with pre-HD individuals. 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-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 hallmark 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 the 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 oestradiol (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 the SN, testosterone increased 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 the SN, testosterone increased sex steroid receptor and steroidogenesis-related mRNAs in the adolescent male rat substantia nigra (SN) and caudate putamen (dorsal striatum).
POS-TUE-181
THE TUMOR SUPPRESSOR PTEN IS TRANSPORTED IN EXOSOMES FOR PHOSPHATASE ACTIVITY IN RECIPIENT CELLS
Putz U., Doan A. and Tan S.-S.

Purpose: Glioblastoma 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 tumour 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 phosphorylation 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 non 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 agonist 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 exercise-induced AHN in female R6/1 mice. Reduced running distances and decreased Akt phosphorylation could underlie the failure of exercise-induced AHN in female R6/1 mice.
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. 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. Atrophy of the ipsilateral hemisphere was evident from 28 days. Cortical and striatal damage varied with different recovery times. Conclusions: This study has documented the evolution of histological damage in the rat post stroke, with delayed recovery times showing more damage. The evolution of histological damage may be due to individual differences in the rate of clean up of damaged tissue to leave a fluid filled cavity, which grows 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-inducively 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 ≤ 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 ≤ 0.001) and degenerated axon profiles (p ≤ 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 cobalt is neurotoxic, the normally high levels of cobalt may contribute to the oxidative stress and demyelination that occur in LTIH. Alternatively, the higher levels of cobalt may indicate that vitamin B12 (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-traumatic 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 ARHGP1A, 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.
POSTERS Tuesday

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 Alzheimer's 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≤0.05). Treatment with 5 μM Amyloid beta1-42 for 24 hours enhanced APP mRNA level (n=6) (p≤0.05), but had no effect on APP protein expression (n=6). We also found that SorLA and BACE1 mRNA were significantly increased in Sortilin expression in SH-SY5Y human neuroblastoma cells and suggesting a potential physiological interaction of Amyloid beta and Sortilin in Alzheimer’s disease. Keywords: Amyloid beta, Sortilin, APP, Alzheimer's 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%O2) in Long Evan rats. In nerve section experiments (n=60) the left median nerve was transected, repaired (using a photochemical bonding procedure) and allowed to recover. In sham experiments (n=20) identical surgery was conducted under anaesthesia (isoflurane 2-5% in 100%02) in Long Evan rats. In nerve section experiments (n=60) the left median nerve was intact. Sensory testing in the distal paws included withdrawal latencies for mechanical and thermal stimuli, and a grasp with the left paw, and grip strength was significantly reduced in FAST (n=3) versus SLOW (n=3) myelin. Results: One week following nerve section, animals were unable to grasp with either 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 stimuli treated 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 analyses 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). We further correlated white matter alterations reported on both the epilepsy 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 significantly 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.
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.

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.

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 (rottenone, 3-nitropipionic acid, antimycin A and KCN, respectively) and drugs that induce PCD: staurosporine (STS, apoptosis) and H2O2 (oxidative stress). 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=3). Inhibition of mitochondrial respiratory complexes induced puncta formation of microtubule-associated protein 1 light chain 3 (LC3-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=3) was interrelated to the concentration-, time-dependent decreases of ATP level under mitochondrial respiratory chain complex inhibition, STS and H2O2 treatment (n=3). This evidence suggests the involvement of respiratory complexes in the recruitment of autophagic mechanisms. Conclusion: The involvement of mitochondrial complex inhibition in autophagy and mitochondrial energetics. Autophagic mechanisms are recruited to PCD by diverse cellular insults including those mediated via respiratory complexes.

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) PA2A are intimately related. We have previously reported in cultured cells and in vivo that folate deficiency induces the loss of methylated PP2A/Bc 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/c clinical consequences of MTHFR C677T polymorphisms in human. Moreover, acute administration 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.
POSTER 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 contributor 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, immuno-labelled with β-III-tubulin and surviving motor neurons counted. Cells were counted in 3mm².

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 (28.0±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 compartmental 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.

POSTER 198

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

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Purpose: Colonic motility and defeation 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 intermittent 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 defeation and soiling in disease 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 defeactions/ wk (meansSEM 1.43±0.6 pre to 4.0±1.5 episodes/wk, p<0.0001) with 32/56 increasing to >3 defeactions/wk. Urge-initiated defeation 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±1.74 to 1.1±1.5 episodes/wk, p<0.0001. Good clinician training and close patient contact were needed. Conclusion: Non-invasive transcutaneous neuromodulation administered at home increased defeation and reduced soiling in STC children. Further studies are required to determine which nerves are affected.

POSTER 199

DIRECT BACTERIAL ENTRY INTO THE BRAIN: BURKHOLDERIA PSUEDOMALLEI AND THE OLFATORY NERVE

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Meliodosis 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: To demonstrate that B. pseudomallei enters the CNS 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 lower 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.

POSTER 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 (6% oxygen, 3 hours) groups. On P7, pups were further separated into HI and sham surgery groups. HI surgery pups were anesthetised with 1.5% isoflurane 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 18.6±2.8% brain injury when compared to controls (n=8; 32.19±7.6% 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±6.81% 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<0.005).

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. Each fetus IUGR was induced via a single umbilical artery ligation. Each twin was implanted with a carotid artery flow probe, electrocorticograph (ECoG) electrodes overlerying 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±4.9% 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 each fetal brain, the number of 4-8HE (lipid peroxidation) positive cells was increased in the cortex of IUGR+BM fetuses (25.9±11.2/mm²) and reduced following MLT administration in IUGR+BM+MLT fetuses (4.9±3.7/mm²). Conclusion: Melatonin does not prevent the rebud carotid blood flow perfusion 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 genes contributing to autism spectrum function are associated with ASD. Neuroligin-3R451C mice express the postsynaptic adhesion protein and show altered GABA-mediated function are associated with ASD. Neuroligin-3R451C mice express the postsynaptic adhesion protein and show altered GABA-mediated synaptic inhibition. These results suggest that the NL3R451C synaptic mutation alters nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility. Nitric oxide released from a subset of enteric neurons (immunoreactive for nitric oxide synthase; NOS) mediates tonic inhibition of colonic motility.

The purpose of this study was to investigate the neuronal subtypes and motility in NL3R451C mice. Some NOS neurons also express GABA/Purinergic receptors. 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, we conducted experiments on day seven; the fetal brain was fixed and processed for light microscopy.

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.
SHORT TERM NEUROPROTECTIVE ACTIONS OF HYPOXIC POSTCONDITIONING IN A NEONATAL RAT MODEL OF HYPOXIC-ISHAEMIC 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 mm³, n=6; CT-PC: -1.47±0.55 mm³, n=8). HI+NC pups had significant injury affecting the ipsilateral hemisphere (-15.67±4.37 mm³, n=14, p<0.05, 1-way ANOVA), and the injury was reduced by PC (HI+PC: -5.15±2.6 mm³, 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-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 models predetermine the preclinical 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 aortic arch 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 tirasaline. 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 dysfunction induced by environmental stress, which increases vulnerability to later 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 plantar withdrawal latency. 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 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. 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 induced 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.
POSIT-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 and temporal neocortical tissue. However, a significant number of TLE patients have no structural lesion identified on MRI, despite hypometabolism on fluorodeoxyglucose-PET (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 GABAergic benzodiazepine receptor binding (with [11C]flumazenil (FMZ) PET) in patients with MTS and non-lesional (NL) TLE.

Methods: FDG and [11C]FMZ PET were acquired in 12 MTS and 19 NL TLE patients with well-localised EEG seizure onsets. Hippocampal volumes, FDG uptake and [11C]FMZ binding were calculated using a 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 (r²=0.63, p<0.001), but not MTS patients (p=0.03, p=0.80, contralateral r²=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 (r²=-0.47, p=0.07), but not the MTS patients (r²=0.03). 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 the MTS patients than in the 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 lobe abnormalities, compared with NL patients who display both mesial and neocortical temporal abnormalities.

POSIT-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 injury sparing 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=8) 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 normal, highly coordinated locomotion (BBB 15±0.8). The BBB index (15±1.6). Axonal tracing revealed that long descending supraspinal axons and propriospinal fibres had grown across the injury site. Consequendy, animals were able to swim and the hindlimbs suggested that supraspinal control had been reestablished. P28-injured Monodelphis recovered weight-bearing use of their hindlimbs but with only limited coordination (BBB 12±0.0). striations continued to swim and were only limited coordination (BBB 12±0.0). Strikingly, the 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.

POSIT-215

KINASE CONTROL OF TDP-43 ACCUMULATION IN MOTOR NEURON DISEASE

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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 Neuronal-epithelial 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 Biotin Dextran, to label CST axons. Results: Multiple kinase inhibitors that target JNK, GSK3 or CK2 were found to prevent abnormal TDP-43 accumulation, including both endogenous and transfected TDP-43. CK2 inhibition was also able to reverse TDP-43 accumulation. Activated forms of JNK, CK2 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 CK2. Moreover, induction of motor neuron hybrid cells with mutated TDP-43 (A315T or Q331K) resulted in abnormal hnrNP K expression and phosphorylation. Inhibitors of JNK, CK2 and GSK3: JNK, CK2 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.
POSTERS

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 hSOD1G93A 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 litters 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). 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 hSOD1G93A mice (n=7-9) and hSOD1G93A 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. hSOD1G93A mice treated with PMX205 showed significantly reduced muscle weakness, and increased survival compared to untreated mice. Similar results were found with CD88-deficient hSOD1G93A mice compared with CD88-sufficient hSOD1G93A 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-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. Hence, we investigated the effect 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 group) provided by the Australian Biobanking Biobank, and immunohistochemistry (n=5/age). We used human microvascular endothelial cells for in vitro cell culture studies, we found that the application of CCL2 (100ng/ml) to microvascular endothelial cells resulted in translocation of the CCL2 receptor, CCR2, out of the cytoplasm and into the membrane. Results: 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. Conclusion: The application of CCL2 to human microvascular endothelial cells results in translocation of the CCL2 receptor, CCR2, out of the cytoplasm and into the membrane.

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 infusion of 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 vivo 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.
**POSTERS**

**POS-TUE-221**

**GENE EXPRESSION PROFILING OF ROTENONE-MEDIATED CORTICAL NEURONAL DEATH: EVIDENCE FOR INHIBITION OF UBQUITIN-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 Ver1.1 arrays. The absolute data was analyzed using GeneSpring GX 7.3. Genes with fold change ± 1.5-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 ≤ 0.05). Results: Global gene profiling analyses revealed a list of nine affected biological pathways. These include programmed cell death, mitochondria dysfunction, cytoskeleton, 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 [57Co]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 [57Co]cyanocbl were lysed and fractionated and [57Co] was measured in lysosomal fractions by gamma-counting. Results: As a percentage of total cellular [57Co]Cbl, fibroblast lysosomal [57Co]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 [57Co]Cbl was ~ doubled to 11.8% of total cellular [57Co]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 a dependent using lysosomal [57Co]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-deoxoadenosyl-Cbl to mitochondrial methionyl-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 conditions that are involved in opiate 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 nalozone-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 type 1 receptor antagonist (SB-334867, 0.2 µL, 100 µM) or nalozone (2 µL) precipitated neurodegenerative diseases 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-deoxoadenosyl-Cbl to mitochondrial methionyl-coenzyme A mutase. Conclusions: These studies provide evidence that age-related lysosomal dysfunction significantly inhibits Cbl transport from lysosomes.

**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 nalozone-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 type 1 receptor antagonist (SB-334867, 0.2 µL, 100 µM) significantly decreases nalozone 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 nalozone 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 RSNs 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 Vs 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-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/kg BW; 5-Pb+vitamin E 100 mg/kg BW and 9-vegetable oil (the same kgBW; 5-Pb+TL 200 mg/kgBW; 6-Pb+TL 400 mg/kgBW; 7-Pb+TL 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 9-0.00±0.03 (mg/mg protein). For blood carbonylation 1-0.05±0.04, 2-32.60±0.67, 3-3.25±0.81, 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-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 groups 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.
POSTERS

Tuesday

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 to explore the mechanism of the antidepressant-like effect of 17beta-estradiol in a mouse model(s) of behavioral despair (depress behaviour). Method: Despair behavior, expressed as helplessness to escape from a situation (immobility period), 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 anitimmobility effect of selective subtypes 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 D2 dopamine receptor antagonist), SCH 23390 (0.5 mg/kg, i.p.; a D1 dopamine receptor antagonist), and sulpiride (5 mg/kg, i.p.; a specific dopamine D2 receptor antagonist) (n=48). In mice pretreated with (+)-pentazocine (2.5 mg/kg, i.p.; a high affinity sigma-1 receptor agonist) and 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 microg/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 serotoninergic 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 LUs 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 (Oxford Optronix). 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 MICRONRNAS 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 peripheral neurodegeneration 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, non-coding RNA molecules, have emerged as new potential regulatory factors that are involved in the modulation of immune responses. 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, miRNA 200b 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 proinflammatory cytokine and iNOS. Conversely, overexpression of miR-200b led to a decrease in the pro-inflammatory cytokine and iNOS expression. Finally treatment of neuronal cells, MIN9 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 therefore affecting their morphology. Conclusion: These results demonstrate the important role of miR-200b in modulating the MAPK pathway via c-Jun 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 rats 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). Conclusion: Our findings suggest that up-regulation of Par-4 in extema 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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**ABSTRACT:**

Hypobaric hypoxia is a mild stress that occurs at high altitude due to a decrease in partial pressure of oxygen. It causes neurodegeneration which further leads to memory impairment. Hypocampus 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 into functional networks 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 were 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 conjunction 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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**ABSTRACT:**

Plantar incision in rat generates spontaneous pain behavior. Morphine used to treat severe postsurgical pain produces tolerance after long term administration. However, analgesics like loperamide, a 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 behavior 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 7 th 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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**ABSTRACT:**

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 upon 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 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 10^5 cells) in ketamine and xylazine (60 and 10 mg/kg, respectively) anesthetized rats, laminectomy followed 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 10^5 cells) 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 ENDOXOSAL DEVELOPMENT DUE TO DEFICIENCY IN SNARES VT1A AND VT1B**

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**ABSTRACT:**

Purpose: The purpose of this research was to identify the function of two SNARE genes i.e. vt1a and vt1b which are involved in early endosomal/trans-Golgi network and late endosomal trafficking events respectively. **Methods:** Double knockout mice (DKO) was used for both vt1a(-/-) and vt1b(-/-) which was compared with double heterozygotes (DHET; vt1a(-/-) vt1b(-/-)). 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) were noted in DKO mice. In CNS, they show wide ventricles and lacked several fiber tracts including anterior commissure, spinotrigeminal, corticospinal tracts, hippocampus as well as nigrostriatal fibers. Corpus callosum thickness was reduced and the thalamocortical axons did not cross palio-subpallial border. While, only a few corticothalamic fibers 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 vt1b(-/-); vt1b(-/-) neurons was suggested by overall phenotype leading to absence or reduction in axonal fiber formation. vt1a(-/-) or vt1b(-/-) single deficient mice were viable without these neuronal defects, indicating that they can substitute for each other in these processes.
CHARACTERISTICS OF PRR2 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.596C>T, c.847T>G, c.596G>A et al.) and truncated PRRT2 (c.849delC, c.514-517delCTCG, 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 father. 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 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.

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 5% 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 NRG1Cre-ERT2 flox flox mice and ErbB4 flox flox 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 picrotoxin-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.

MEC-17 DEFICIENCY LEADS TO REDUCED a-TUBULIN ACETYLACTION 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 a-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 a-tubulin acetyltransferase that has been found to play a major role in the acetylation of a-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 disrupted the transition of projection neurons from the multipolar stage to the unipolar/striatal stage in the intermediate zone of the cortex. Furthermore, knockdown of a-tubulin deacetylase HDAC6 or overexpression of tubulin acetylation resulted in disrupted or disturbed cortical projection neurons. Thus, MEC-17, which regulates the acetylation of a-tubulin, appears to control the migration and morphological transition of cortical neurons. This finding reveals the importance of MEC-17 and a-tubulin acetylation in cortical development.

A NOVEL SYNTHETIC CURCUMINOID DERIVATIVE ATTENUATES NEUROPATHIC PAIN IN MICE VIA NO-INDEPENDENT cGMP ACTIVATION OF KATP

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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 (KATP) pathway in anti-hyperalgesic 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. The effect of BHMC on CCI-induced neuropathic pain was evaluated using mechanical Randall Sellito test. CCI-induced pain (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 KATP opening, leading to hyperpolarisation in nociceptive neurons. Further dissection of the mechanism of analgesic action of BHMC is required to establish BHMC as prospective analgesic agent clinically.
**POSTERS**

**POS-TUE-241**

**MONTELUKAST MODULATES THE PROTECTIVE EFFECT OF ROFECOXIB AND CAFFEIC ACID AGAINST KAICIN 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) against kaicin acid-induced cognitive dysfunction in rats. Methods: In the experimental protocol, kaicin acid (0.4µg/2µl) in Artificial 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 post-injury. 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 (kaicin acid treated), however, combined treatment 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 (cyclo-oxygenase) and LOX (lipoxigenase) pathways in the control of the neuroinflammation in kaicin acid induced cognitive dysfunction in rats.

**POSTERS**

**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-Ki (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 multicellular aggregates, embryoid bodies (EBs), suggesting the wider differentiation spectrum of the cells including neural lineages. In this experiment, we aim to explore 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 (Neurilament, MAP2, GFAP and TH). Results: Upon 2-hours of induction, rat full term AFSCs showed early neuronal marker (nestin and PAX-6). After 2 days, beta tubulin III was detected. GFAP expression was observed from day 4 to day 10. Neurilament, 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.

**POSTERS**

**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. Activated microglia are known to secrete extreme amounts of reactive oxygen species (ROS), causing oxidative stress thereby resulting in neurodegeneration. Sirtuin 3 (Sirt3), a member of the protein deacetylating enzyme, mediates the antioxidant defense mechanism and protects tissues from oxidative damage and the expression was upregulated on the 15th day post-injury. 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 (kaicin acid treated), however, combined treatment 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 (cyclo-oxygenase) and LOX (lipoxigenase) pathways in the control of the neuroinflammation in kaicin acid induced cognitive dysfunction in rats.

**POSTERS**

**POS-TUE-244**

**INFLUENCE OF FORCED SWIM STRESS ON NEUROBEHAVIORAL TOXICITY OF LAMBDA- CYHALOTHRON 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 associated with decreased neurobehavioral toxicity of lambda-cyhalothrin. The results exhibit improved learning and memory compared to controls. Increased levels of plasma corticosterone associated with decreased plasma corticosterone associated with decreased binding of cholinergic-muscarinic receptors and protein expression of CHAT 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 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 BX 30 in the control group. Conclusion: The results exhibit that physical stressor (FSS) could be a contributing factor in enhanced neurobehavioral toxicity of lambda-cyhalothrin.
POSTERS

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 immunohistochemical analysis. The cell survival enhancing property of WS was studied using expression of BAX, Caspase 8, and Akt along with immunohistochemical 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 MITOCHOME 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 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 possesses 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 extracts 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 glutal fibrilary acidic protein (GFAP) expression in differentiated C6 cells. Results: Methanol Extract (CME) significantly decreased the SOSIP of H2O2 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 (KARNAKATA) 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 immunohistochemical techniques. Results: In the logistic regression analysis, increasing age was associated significantly on AD related (Braak, CERAD & CAA) pathologies. Population and sex factors did not 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 frontal temporal regions-spongios [odds ratio (OR)=5.90, 95% CI=2.981-24.279], dilaated 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. FGF13 promotes 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 anodromous 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 sensory brain regions. Results: The right cerebral ventricle (10µg/10µli.c.v.) immediately before morphine showed the development of tolerance to morphine at the cellular level. Results: Administration of SB-334867 before each morphine injection reversed a significant decrease in responses of LPGi neurons to morphine. Conclusion: 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-252

OREXIN TYPE-1 RECEPTOR MEDIATES THE DEVELOPMENT OF TOLERANCE TO MORPHINE IN LATERAL PARAGIGANTOCellularIAR 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 Ab1-42, 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<0.002) were significantly associated with a CSF-AD like profile. Having a CSF-AD profile was associated with lower current neurocognitive performance (p<0.002). 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 CORICAL 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 Parkinson’s disease relevant factors.

Methods: In this study, we investigated the dose responses of adult rat neurons to various concentrations of α-Syn isofoms. 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 two monocular contrasts equaled 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 24hr 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<0.05). There was no change in locomotor activity across treatments. Furthermore, 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 OVEREXRESSING 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 an axon terminal 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 mortality and in behavioural deficits. Performance on the rotarod task decreased significantly from a pre-injury 29.3±3 rpm to 22.9 rpm in the 5.15 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 in more severe brain damage. Furthermore, age played a major role in mortality, but no weight effect was observed (which age and impact velocity were 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, 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 were 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.


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.

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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Purpose: 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.

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 frontal 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.