POSTERS

POS-WED-001

CELL DIVISION IN DEVELOPING MOUSE SYMPATHETIC GANGLIA: THE ORIGINS OF NEURONS AND GLIA

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Sympathetic ganglia develop from neural crest (NC) cells that migrate to the site of the sympathetic ganglia and undergo proliferation and differentiation into both neurons and glia. To identify the peaks times of cell division for neurons and non-neuronal cells, we injected BrdU into pregnant dams (n=14) 2h prior to fixing the embryos. We examined embryonic stellate ganglia between embryonic day (E) 11.5 and birth (P0). Immunoreactivity (IR) to tyrosine hydroxylase (TH) and Sox10 was used to identify cells destined to become neurons and non-neuronal cells respectively. At E11.5, 87% of the cells in the ganglion were TH-IR and 13% were Sox10-IR. BrdU-IR was present in about half of both groups of cells, indicating they were dividing at a similar rate. Nearly half the Sox10-IR cells showed co-localised immunoreactivity to TH, suggesting that they were differentiating from NC cells into neurons. By E13.5 the proportion of cells that showed TH-IR had increased to 90% of the total and BrdU-IR was present in only 25% of TH-IR cells and 17% of Sox10 cells. Very few Sox10 cells showed colocalised TH-IR at this stage. By E14.5 TH-IR cells were still close to 90% of the total, but the proportion of both types of cells with BrdU-IR was decreased. By E16.5 there was little or no BrdU-IR present in TH-IR cells and the proportion of Sox10-IR cells dividing was increased. By P0 approximately 30% of the cells in the ganglion express Sox10. We believe the expansion of the number of neurons in the ganglion after E11.5 is driven by the division of sympathetic neurons, rather than the differentiation of Sox10-IR precursor cells.

POS-THU-002

WORMING OUR WAY TOWARD AXONAL DEGENERATION AND REGENERATION

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Axonal degeneration is a characteristic phenotype in many neurological disorders, including ALS, Parkinson's and Alzheimer's diseases, and often precedes the death of the neuronal cell body. However, the genetic mechanisms behind axonal degeneration are poorly understood. The nematode *C. elegans*, with its simple nervous system (made of only 302 neurons), short life cycle and genetically favorable characteristics, is an attractive model system for the study of this important biological process. The aim of our research is to identify with a genetic approach the key molecules and mechanisms responsible for axonal degeneration. Using a transgenic line expressing GFP in the mechanosensory neurons, we have carried out a mutagenesis screen and identified mutant animals displaying spontaneous axonal degeneration in a subset of these cells. Within *C. elegans* strain CX850, the neuronal processes of PLM neurons begin fragmenting and degenerating in the adult stage and progressively worsen over time. As expected, these animals are rather insensitive to mechanical touch when compared to their wild-type controls, indicating that the axonal degeneration observed is affecting normal neuronal function. The next stage of our research is to identify the mutation responsible for the axonal degeneration phenotype observed within the CX850 strain. A complementary and equally important process is axonal regeneration, defined as the active regrowth of the proximal fragment (the one attached to the cell body) in a severed axon. Using a UV laser, we are able to perform axotomies on individual *C. elegans* neurons and analyse their regeneration dynamics. Several *C. elegans* neurons are able to regenerate and restore function after axotomy. Using different transgenic lines and a candidate mutant approach, we are now investigating the dynamics of the axonal membrane and the genes regulating this dramatic and important process. We hope that these studies will improve our current understating of nerve degeneration and regeneration, providing new insights toward the development of more effective therapies for neurodegenerative diseases and spinal cord injuries.

POS-WED-003

EFFECTS OF ENVIRONMENTAL ENRICHMENT ON MOUSE BEHAVIOUR AND STRIATAL PLASTICITY

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Purpose: Environmental enrichment can elicit widespread changes in rodent behaviour, neuroanatomy and neurophysiology. However, most studies have explored only post-weaning enrichment (from post-natal day (P) 21), and few have investigated its effects on subcortical structures, such as the striatum, which is central in determining learning and behaviour. The striatum exhibits age-related changes in density and distribution of perineuronal nets (PNNs), extracellular matrix structures implicated in synaptic plasticity. We aimed to examine the effects of pre-weaning enrichment (i.e. from birth) on mouse behaviour and striatal plasticity. **Methods:** C57/BL6 mice were assigned to 1 of 4 conditions: no enrichment (NN), enrichment post- but not pre-weaning (NE), enrichment pre- but not post-weaning (EN) and enrichment from birth (EE). The enriched environment consisted of a larger cage, more mice per cage, and a variety of novel objects and toys, including an exercise wheel, to provide multimodal stimulation. Adult mice (NN, n=11; NE, n=18; EN, n=6; EE, n=14) were subjected to the Morris water maze (MWM; a swimming spatial memory task; 7 days of 4 trials). Sections of adult striatum (all groups: n=3) were labelled for PNNs. **Results:** NE, EN and EE mice had significantly lower MWM latencies than did NN mice across all trials (NN vs NE, $p<.001$; NN vs EN, $p<0.05$; NN vs EE, $p<0.01$). Qualitative assessment consistently yielded the presence of PNN-empty regions in specific striatal subregions of EE sections. **Conclusion:** Enrichment improves adult MWM performance, regardless of the recency of exposure. The effects of enrichment on behaviour and the nature of striatal plasticity of the developing mouse (P10) are currently being investigated.

POS-THU-004

MATERNAL CIGARETTE SMOKE EXPOSURE DOWNREGULATES HYPOTHALAMIC NPY AND Y1 RECEPTOR IN OFFSPRING

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In humans, maternal smoking leads to increased risk of obesity in offspring. Nicotine passes rapidly and completely across the placenta, with fetal concentrations reaching 15% above maternal levels. How this may affect the neuroendocrine mechanisms involved in energy homeostasis is poorly understood. We hypothesized that maternal smoking alters hypothalamic appetite regulators such as the appetite stimulator neuropeptide Y (NPY), to promote hyperphagia and adiposity in offspring, which would be exaggerated by maternal obesity. Female Balb/c mice (7week) were either cigarette smoke exposed (SE, 2 cigarettes/day, 5 days/week) or sham exposed for 5 weeks before mating. Across groups, half were fed high-fat diet (HFD, 33% fat) versus chow control throughout gestation and lactation. Female offspring were weaned onto chow and sacrificed at 12 weeks. Birth weights were similar across maternal groups. At weaning pups from SE+chow, SE+HFD and sham+HFD mothers were 15%, 26%, and 36% heavier than those from sham+chow mothers, respectively. At 12 weeks, offspring from HFD-fed mothers were significantly heavier than those from chow-fed mothers (chow+sham 17.6 ± 0.3g; chow+SE 17.8 ± 0.2g; HFD+sham 18.7 ± 0.3g; HFD+SE 18.8 ± 0.4g, $P< 0.05$), and fat mass was significantly greater in offspring from SE+chow, SE+HFD and sham+HFD mothers, with slightly lower energy intake. Plasma leptin concentrations were only significantly higher in pups from SE+chow mothers. Hypothalamic NPY mRNA was lower in offspring from HFD+sham and HFD+SE mothers and Y1 receptor mRNA was reduced in offspring from all the treated maternal groups. Thus both maternal SE and HFD consumption reduced hypothalamic NPY signaling. However, this did not prevent the increased adiposity in these animals. Maternal smoking cessation is desirable to maintain energy homeostasis.

POS-WED-005

POTENTIAL ROLES FOR MULTIPLE TENEURIN MOLECULES IN THE REGULATION OF CIRCUITRY IN THE MOUSE VISUAL PATHWAY

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Purpose: The Teneurin (Ten-m/Odz) family comprises four highly conserved, homophilic transmembrane glycoproteins. Previous work from our lab has shown that Ten-m3 plays an important function in regulating development of the binocular visual pathway in mice. This study investigated potential roles for other members of the Ten-m family in regulating visual connectivity. **Methods:** Riboprobes for Ten-m1, Ten-m2, and Ten-m4 were synthesised and *in situ* hybridisation was performed in postnatal day (P)0-P7 mice. Organisation of retinal projections to the dorsal lateral geniculate nucleus (dLGN) was assessed with intraocular injections of cholera toxin B conjugated to red or green fluorescent dyes in Ten-m2 knockout (KO) and wildtype (WT) littermates (P27-30). **Results:** Ten-m2 and Ten-m4 are expressed in the dLGN from P0. Ten-m2 appeared to be graded with highest expression dorsolaterally. No distinct gradient was observed for Ten-m4. Ten-m2 and Ten-m4 were both expressed in visual cortex. Ten-m1 expression was not observed in the dLGN although high expression was observed in the adjacent auditory thalamus. Ten-m2 KO mice displayed alterations in the organisation of retinal projections. Most notably, ipsilateral projections occupied a significantly ($p<0.01$, t-test) lower proportion of dLGN area over almost the entire rostral half of the nucleus in KOs (n=8) compared to WTs (n=7). **Conclusion:** Together, these data show that multiple members of the Ten-m family are expressed in the developing visual pathway and play regulatory roles in the development of visual connectivity. Intriguingly, Ten-m2 and Ten-m3 both regulate development of binocular pathways but play distinct, complementary roles in this process.

POS-THU-006

ERYTHROPOIETIN PROTECTS THE DEVELOPING RETINA AND OPTIC NERVE FROM INJURY INDUCED BY EXPOSURE TO ENDOTOXIN?

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Purpose: Intrauterine infection is considered to be a cause of preterm birth and brain injury; visual dysfunction occurs in some very preterm infants. We investigated the neuroprotective effects of recombinant human erythropoietin (rhEpo) in the retina and optic nerve in an ovine model of fetal inflammation induced with the endotoxin lipopolysaccharide (LPS). Methods: Catheterised fetal sheep in utero underwent one of four intravenous treatments (saline, n=9; LPS (0.9ug/kg); n= 6; LPS+rhEpo (5000 IU/kg) administered one hour after LPS, n=8; Epo, n= 5) on three consecutive days from 105 days of gestation (dg; term = 147 dg). At 115dg, myelinated axons in the optic nerve and tyrosine hydroxylase-immunoreactive (TH-IR) dopaminergic amacrine cells in the retina were assessed quantitatively. Dopaminergic amacrine cells are interneurons known to be essential for normal retinal function and are particularly sensitive to prenatal insults. Results: The number of TH-IR cells was lower in LPS-exposed fetuses than in saline-treated controls ($p<0.05$) but was not different from controls in LPS+rhEpo-treated fetuses ($p>0.05$). The total number of myelinated axons was lower in the LPS-exposed fetuses than in saline-treated controls ($p<0.05$), but was not different from controls in LPS+rhEpo-treated fetuses. Conclusion: This study has shown that rhEpo treatment may be beneficial in protecting developing dopaminergic amacrine cells and myelinated optic axons following LPS-induced inflammation in the immature ovine fetus. The underlying mechanisms are not known but protection could occur via Epo's anti-apoptotic or anti-inflammatory actions. The clinical relevance of Epo is promising but needs to be further assessed.

POS-WED-007

MOLECULAR MECHANISMS UNDERLYING ACTION OF AN EYE SPECIFIC AXON GUIDANCE MOLECULE, TEN-M3

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Purpose: Previous work from our lab has shown that Ten-m3 plays a critical role in generating aligned binocular connections; Ten-m3 knockout (KO) mice display profound visual deficits as a result of mapping errors of ipsilateral retinal projections in the thalamus and superior colliculus (SC). This study aims to identify the molecular mechanisms underlying the loss of visual function in Ten-m3 KO mice. Methods: We have used *in situ* hybridisation to examine changes in the expression of genes with well-established roles in development of the retinocollicular pathway, and microarray analysis of KO versus wildtype (WT) retina and SC using the Affymetrix MoGene 1.0 ST array to identify novel targets of Ten-m3 signalling. Results: Analysis of normalised microarray data identified 258 genes in the SC, and 697 genes in the retina which are differentially expressed in Ten-m3 KO mice compared to WT (Fold Change ≥1.25, t-test $p\leq0.05$; n=3 KO and 3 WT). Expression levels of a number of genes associated with the Hedgehog signalling pathway were changed, suggesting that Ten-m3 interacts with this important morphogenic pathway. Members of the Fgf and Wnt families were also altered. *In situ* wholemount preparations of SC (n=6 KO and 6 WT) showed alterations in Eph/ephrinA expression, with markedly lower levels of EphA7 observed in KOS. Conclusion: Expression levels of a large number of genes are significantly altered in Ten-m3 KOs, consistent with the proposal that the intracellular domain of Ten-m3 acts as a transcription factor. The identification of the Hedgehog pathway as a potential mechanism of action suggests that Ten-m3 may not only regulate axon guidance, but also modulate fundamental events in brain patterning.

POS-THU-008

ALTERED DENDRITIC MORPHOLOGY IN RETINA OF MICE LACKING EPHRIN-A

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Purpose: In mice lacking ephrin-A axon guidance cues (ephrin-A-/-), projections from retinal ganglion cells (RGCs) to the superior colliculus (SC) are mapped abnormally. These topographic errors have been attributed to the absence of ephrin-A2 and -A5 in target tissue, resulting in a lack of guidance information. However ephrin-As have also been reported to play a role in dendritic development and synaptogenesis. Ephrin-A2 and -A5 are expressed in RGCs and it is possible that, in ephrin-A-/- mice, intra-retinal circuitry is abnormal, potentially contributing to the development of aberrant retinocollicular maps. Here we examined RGC dendritic morphology in mice lacking ephrin-A2, and mice lacking both ephrin-A2 and ephrin-A5. **Methods:** Live adult mouse retinas (WT: n=10; A2-/-: n=14; A2/A5-/-: n=11) were wholemounted and fluorogold labeled RGCs injected iontophoretically with 2% lucifer yellow. Cells (n=269) were manually traced using Neurolucida. Values were analyzed separately for the four main RGC types. **Results**: There was no significant difference in the distribution of each cell type between genotypes, suggesting an unbiased sample. Ephrin-A2-/- and ephrin-A2/A5-/- mice showed similar morphological differences compared to WT. A-type RGCs in all retinal locations had longer dendrites and increased field size. B-type and C-type RGCs in knockout mice had smaller cell body areas. C-type RGCs had relatively fewer branch points in nasal retina and more in temporal retina. **Conclusion**: Results suggest that ephrin-A2 plays a role in regulating soma size and dendritic morphology in a cell-type specific manner, while ephrin-A5 has no obvious additive effects. If morphological changes are reflected in functional changes, abnormal retinal circuitry may contribute to errors in retinocollicular topography in ephrin-A-/- mice.

POS-WED-009

THE ROLE OF EPH/EPHRINS IN THE DEVELOPMENT OF VISUAL AREAS IN THE PRIMATE BRAIN

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Purpose: In this present study we demonstrate the involvement of the Eph/ ephrin receptor tyrosine kinase guidance molecules in the development of several of the primate visual cortex, and their unique expression profiles. **Methods:** Coronal and sagittal sections from the brains of marmoset monkeys (Callithrix jacchus) aged ED 115 (before cortical lamination is complete; n=1), PD 0 (n=2) and 12 months (adult; n=2) were processed immunohistochemically for Eph/ ephrin expression. Nissl substance and non-phosphorylated neurofilament (NNF) expression were used to assist in the demarcation of laminar and areal boundaries. **Results:** During postnatal development we observed specific spatiotemporal profiles of expression in a number of the Eph/ ephrins studied. At PD 0, ephrinA5 expression was observed in layers 4 and 6 of the primary visual cortex (V1), especially on NNF-immunopositive pyramidal neurones, which disappeared at both the dorsal and ventral borders with the second visual area V2, where a higher level of expression was observed in layer 3. EphA4 was expressed on the radial processes of the astrotrocytes, which guide the neurones to their target layer. This expression changed to neuronal expression in adult brain. EphA3 was expressed on mature oligodendrocytes during development and maturation and colocalised with CC1 marker. A differential expression was also observed between different cellular layers of the lateral geniculate nucleus (LGN), where strong ephrin A5 expression was only observed in the two magnocelullar layers. **Conclusion:** This study demonstrates that different Eph/ ephrins are expressed during visual cortex development and are important for neuronal guidance during development and formation of cortical boundaries, and become downregulated as the brain matures.

POS-THU-010

THE EFFECT OF PUBERTAL TESTOSTERONE ON BRAIN DEVELOPMENT IN ADOLESCENT RHESUS MACAQUES

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Purpose: Little is known about the effects of sex steroids on the anatomy of the developing primate brain during maturation. **Methods**: To test the hypothesis that testosterone impacts volumes of brain structures during adolescence, T1-weighted structural MRI scans of male rhesus monkeys (*Macaca mulatta*) were obtained before the pubertal surge in testosterone (2.5 years of age) and after the surge (3.5 years of age). Half the monkeys were gonadectomized before puberty (n = 6) while the remainder underwent sham surgery. Borders of the total brain, lateral ventricles, prefrontal white matter and amygdala were manually traced and the volumes estimated. **Results**: Total brain volume for both gonadectomized and intact control monkeys significantly decreased across the study by 5 percent, while white matter volumes in the frontal lobe increased by as much as 20 percent. Importantly, ventricular volume decreased among the intact monkeys while it increased among the gonadectomized monkeys ($p < 0.05$). At this stage in the analysis, we were not able to detect significant changes in the amygdala volume across the time points or between the treatment group. *In situ* hybridization confirmed the expression of androgen receptors in multiple brain regions with increased expression along the borders of the ventricles. **Conclusion**: The results are consistent with previous reports that adolescence involves increases in white matter alongside small decreases in grey matter volume. Furthermore, pubertal testosterone appeared to cause a decrease in lateral ventricle possibly by maintaining or even increasing brain tissue volume. Tissue adjacent to the ventricles, which are a site of rich androgen receptor expression, may be particularly impacted by changes in testosterone levels.

POS-WED-011

THE P75 NEUROTROPHIN RECEPTOR MEDIATES HIPPOCAMPAL CELL DEATH THROUGH G-PROTEIN ACTIVATED INWARDLY RECTIFYING POTASSIUM CHANNELS

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The p75 neurotrophin receptor (p75NTR) is an important initiator of apoptosis in the developing nervous system as well as adult neurodegeneration. We have recently shown a novel p75NTR-initiated cell death pathway involving potassium (K+) efflux through activation of G-protein coupled inwardly rectifying K+ (GIRK) channels. This pathway was established in a heterologous expression system with the current study being designed to establish whether it also exists in neurons. Embryonic day 18 mouse hippocampal neurons were cultured in the presence of amyloid beta 1-42 peptide (Aß1-42), a potent activator of p75NTR-mediated cell death. Neuron survival was significantly reduced in the presence of Aß1-42 compared to the control peptide amyloid beta 1-16 (Aß1-16). This neuronal death could be rescued by addition of the GIRK channel blocker tertiapin, providing the first evidence that endogenous GIRK channels participate in p75NTR induced death of embryonic hippocampal neurons. In addition the GABAb agonist baclofen promoted neuron survival which we hypothesise could occur by establishing normal regulation of GIRK channels. Elucidation of the regulatory components of the p75NTR cell death pathway may provide therapeutic targets for intervention in neurodegenerative diseases such as stroke, Alzheimer's and motor neuron disease.

POS-THU-012

DEVELOPMENT AND PATTERNING OF THE COMMISSURAL PLATE IN MICE

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The specific anatomical positioning of the forebrain interhemispheric commissures at the Commissural plate (CoP) suggests that disrupted molecular patterning may underlie human callosal dysgenesis. **Purpose:** To characterise the development of the CoP in mice. **Methods:** Diffusion tensor magnetic resonance imaging in embryonic mice revealed that commissures crossed the CoP at an oblique coronal angle. Immunohistochemistry detailed the development of the CoP using the growing axon marker GAP43, pallial markers such as Empty spiracles homeobox 1 (EMX1) and Nuclear factor I/A (NFIA), and subpallial markers including Sine oculis-related homeobox 3 homolog (SIX3) and zinc finger protein of the cerebellum 2 (ZIC2). **Results:** Anatomical delineation of CoP into the dorsal Massa Commisuralis (MC) and ventral Area Septalis (SA) was made according to work in human fetal brain by Rakic and Yakovlev (1968). In addition to the corpus callosum (CC), the MC in mouse also contained dorsal and ventral components of the hippocampal commissure (HC). The MC could be further divided into two regions, one expressing EMX1 and NFIA and the other expressing NFIA and ZIC2. The SA could also be divided into two molecular regions based on the expression pattern of SIX3. In Nfia knockout mice, a substantial expansion of the molecular boundary of the MC, but not the SA, was found compared to control (~360 µm; $P < 0.01$, ANOVA; n=3), which correlated with agenesis of the CC and HC. In **conclusion**, the similarities between human and mouse CoP anatomy suggests an evolutionary conservation of this region and establishes a framework for understanding forebrain midline commissure dysgenesis.

POS-WED-013

FGF8 EXPRESSED BY THE COMMISSURAL PLATE GUIDES HIPPOCAMPAL AXONS

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Highly specific anatomical positioning of the forebrain commissures within the Commissural Plate (CoP) suggests this region may express molecules that guide forebrain commissural axons across the midline. One candidate molecule expressed in the CoP is Fibroblast growth factor 8 (FGF8). **Purpose:** To elucidate the role of the CoP and FGF8 in forebrain commissure formation in mice. **Methods:** Immunohistochemistry was used to characterise both the gross morphology and cellular detail of the mouse CoP. The developmental expression of Fgf8 and its receptors was analysed by quantitative real-time PCR. An in vitro guidance assay was utilised to assess the functional effect of recombinant FGF8 on cingulate cortex and hippocampal neuron projections. **Results:** Characterisation of the mouse CoP demonstrated that it is made up of neurons and glia and contains five major axon tracts. Our expression analyses indicate that Fgf8 is expressed in the CoP at E14 then significantly downregulated at E15 ($p \leq 0.05$, Student's t-test) and E16 ($p \leq 0.05$, Student's t-test; n = 3 for all conditions). Recombinant FGF8 protein induced significant attractive guidance of hippocampal neuron projections ($p \leq 0.05$, Student's t-test; n = 76, control, and n = 78, FGF8 cultures), but not cingulate cortex neuron projections ($p = 0.18$, n = 76, control and n = 78, FGF8 cultures). This result correlated with higher Fgfr3c expression in the hippocampus than in the cingulate cortex ($p \leq 0.05$, Student's t-test; n = 3 for all conditions). **Conclusion:** These findings suggest that FGF8 expressed by the CoP may be an important guidance cue for hippocampal commissural projections at the interhemispheric midline.

POS-THU-014

POSSIBLE GUIDANCE MECHANISMS IN THE CAUDAL CORPUS CALLOSUM

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Purpose: The corpus callosum is the largest fibre tract in the brain connecting neurons in the left and right cerebral hemispheres. The mechanisms that guide axons across the midline in rostral regions of the corpus callosum are well understood but little is known about the guidance mechanisms in the caudal region. The hippocampal commissure is thought to provide midline guidance to pioneering callosal axons from the cingulate cortex. The development of the caudal region of the corpus callosum was investigated in mouse by analysing the growth parameters of the corpus callosum from birth to adult and by investigating the presence of midline cell populations that may be involved in callosal axon guidance. **Methods:** Growth parameters were determined by ImageJ analysis of gold chloride stained mouse brains from P0 to Adult. Immunohistochemical studies (GFAP, Calretinin, Gap-43, Caspase-3, and Cytokeratin) characterised morphological features in coronal serial paraffin sections of mouse brain from P0 to Adult. **Results:** The majority of growth in the caudal region of the corpus callosum occurred after birth (n=3). Exuberance and pruning of axons occurred along the entire rostral-caudal axis. Midline cell populations were identified in the caudal region similar to those known in the rostral region. Axons in the most caudal region of the corpus callosum crossed the midline independent of the hippocampal commissure and independent of a glial substrate (n=3). **Conclusion:** Similar midline guidance mechanisms exist in the developing rostral and caudal corpus callosum while the hippocampal commissure does not appear to provide callosal guidance in the caudal corpus callosum.

POS-WED-015

NETRIN1 AND DCC IN CORPUS CALLOSUM FORMATION

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PURPOSE: Previous studies have shown that Netrin1 and DCC mutant mice have defects in the formation of spinal cord commissural projections, the optic chiasm and all forebrain commissures (Serafini et al., 1996; Fazeli et al., 1997). Netrin1 attracts developing corticofugal axons through the internal capsule (Richards et al., 1997; Metin et al., 1997, Braisted et al., 2000). Here, we investigated the hypothesis that Netrin1 attracts callosal axons to the midline. **METHOD**: The forebrain phenotype of Netrin1 and DCC mutant mice was examined using tract tracing and immunohistochemistry. A collagen gel assay was used to compare the attractive response of axons from neocortical explants derived from earlier and later stages of neocortical development in both rats and mice. **RESULTS**: As previously shown, axons from earlier stage (E15 rat) neocortical explants were attracted to Netrin1 (n=85 control explants and n=94 netrin1 explants from 4 separate experiments; $p<0.001$, Student's t-test). However, axons from later stage (E19) neocortical explants showed no response (n=141 control explants and n=153 Netrin1 explants from 4 separate experiments; $p=0.072$, Student's t-test), indicating that the primary role of Netrin1 in corpus callosum formation may not be as an attractant for neocortical axons. Analysis of Netrin1 and DCC mutant brains showed additional defects in midline glia, subcallosal sling formation, midline fusion and disruptions in the formation of the callosal pioneering axons from the cingulate cortex (n=3 for each genotype). **CONCLUSION**: Our results suggest that Netrin1 and DCC may regulate multiple processes in midline commissure formation in the brain, but that they do not regulate the attraction of callosal axons from the neocortex.

POS-THU-016

LOCALISATION AND EXPRESSION OF COMPLEMENT C5A RECEPTORS IN A MOUSE MODEL OF MOTOR NEURON DISEASE

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Purpose: C5a is an inflammatory molecule generated upon activation of the complement cascade, which has been recently implicated in motoneuron disease (MND). This study examined the expression and localization of the receptors for C5a, C5aR and C5L2, in a mouse model of MND. **Methods:** C57BL/6J SOD1G93A transgenic mice and their wild type (WT) littermates were examined at 3 different post-natal (P) ages: P30 pre-symptomatic; P70 onset of MND; and P160 MND end stage. At each age, the localization of C5aR and C5L2 within the spinal cord was determined by immuno-histochemistry (n=3/age). This was performed using antibodies to these receptors along with molecular markers for neurons and glia. Expression levels of these receptors were then investigated by Western blotting (n=3/age). **Results:** C5aR was localized to motoneurons in WT and SOD1G93A mice at P30. As SOD1G93A mice aged and displayed MND symptoms at 70 to 160 days, C5aR was found to be expressed on motoneurons and proliferating microglia, whereas C5aR only remained on motoneurons in WT mice at these ages. This increase in C5aR localization led to an increase in C5aR protein expression at P160. C5L2 immuno-staining was localized to motoneurons in both WT and SOD1G93A mice at all ages. Western-blotting of C5L2 revealed that at P30, protein expression in SOD1G93A mice was significantly higher compared to WT mice. Thereafter, C5L2 protein levels were no different between WT and SOD1G93A mice (P70 and P160). **Conclusion:** These results indicate that C5a and its receptors are involved in the progression of MND in the SOD1G93A mouse model.

POS-WED-017

OLFACTORY ENSHEATHING CELLS AND SCHWANN CELLS INTERACT WITH GLIAL CELLS AND NEURONS VIA HIGHLY MOTILE LAMELLIPODIAL WAVES

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Implantation of olfactory ensheathing cells (OECs) or Schwann cells (SCs) into damaged CNS tracts have led to axonal regeneration but the results are not optimal and the favorable glial type may vary depending on the site of injury. Therefore it is crucial to investigate the functional differences between OECs and SCs in terms of modulation of different types of neurons, and to determine how these glial cells interact with neurons. We have previously shown that OECs display highly motile peripheral membrane protrusions termed lamellipodial waves, which mediate cell-cell contact between OECs and may potentially also mediate OEC-axon contacts. Using high-speed, high-resolution time lapse imaging of fluorescently labeled cells, we have for the first time demonstrated the presence of dynamic lamellipodial waves in SCs. The waves were similar to those in OECs in terms of number of waves/cell and wave area (n = 14-15) but traveled ~2-fold faster (n = 20; $p \leq 0.01$, t-test). Both OECs and SCs interacted directly with DRG axons via lamellipodial waves. Finally, we showed that both SCs and OECs promote survival of DRG neurons. In a nutrient-free medium, neuronal survival after 3 days was increased from 40 % to 67 % by SCs ($p \leq 0.05$) and to 77 % by OECs ($p \leq 0.01$) (n = 8-16 wells containing ~200 neurons; one-way Anova, Tukey's post test). There was no significant difference between OECs and SCs, suggesting that transplantation of both glial types may promote regeneration of damaged sensory neurons.

POS-THU-018

LIVE CELL IMAGING REVEALS THAT OLFACTORY ENSHEATHING CELLS ARE A HETEROGENOUS POPULATION OF CELLS WITH FUNDAMENTAL DIFFERENCES IN THEIR BEHAVIOUR FOLLOWING CELL-CELL CONTACT

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Purpose: Transplantation of olfactory ensheathing cells (OECs) derived from the peripheral and central nervous system produce different outcomes when used for neural regeneration therapies. However, the anatomy suggests that OECs from different regions of the olfactory system will have different functions. Understanding the mechanisms underlying the interactions of different subpopulations of OECs may lead to therapeutic improvements. **Methods:** We have used live cell time-lapse imaging to analyse the behaviour during cell-cell interactions of different subpopulations of OECs from several developmental ages. **Results:** We have found that OECs from the peripheral olfactory nerve (n=53) are a homogeneous population of cells that respond uniformly to cell-cell contact with the vast majority of their interactions resulting in cell adhesion. In contrast, OECs from the olfactory bulb are a heterogeneous population of cells. OECs from the rostral olfactory bulb (n=50) display an even mix of adhesion, repulsion and cross-over in response to cell-cell contact; and they maintain these responses throughout development. In comparison, OECs from the dorsal (n=59), ventral (n=60) and caudal (n=51) olfactory bulb also display a mix of responses but these responses change with increasing developmental age. **Conclusion:** We report here that peripheral and central derived OECs display fundamental differences in behaviour during cell-cell interactions. The manipulation of specific behaviours of subpopulations of OECs may lead to the enhancement of therapeutic outcomes in OEC mediated neural repair.

POS-WED-019

NEONATAL LIPOPOLYSACCHARIDE EXPOSURE ATTENUATES THE ADULT FEBRILE RESPONSE IN MALE BUT NOT FEMALE WISTAR RATS

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Purpose: The adult systemic inflammatory response is susceptible to modification by early-life infection. This is proposed to be mediated by alterations to the hypothalamic-pituitary-adrenal (HPA) axis. This study investigated whether neonatal exposure to a bacterial stimulus alters the acute phase response (i.e., fever, immune and endocrine responses) to an immune challenge in adulthood. **Methods:** Neonatal Wistar rats (n = 57) were administered *Salmonella enteritidis* lipopolysaccharide (LPS, 0.05 mg/kg, ip) or saline (equivolume) on days 3 and 5 of life. In adulthood, subjects were administered LPS (0.10 mg/kg, ip) or saline (equivolume), and the febrile response was monitored biotelemetrically for 24 hours. A different group of rats (n = 29) received identical neonatal treatment, and peripheral blood was collected for corticosterone analysis at baseline, 30, 60, 90 and 180 minutes following adult LPS or saline administration. At 180 minutes following the adult treatment, the animals were perfused with saline and the hypothalamus was collected for the determination of IL-1β levels. **Results:** Male but not female rats neonatally exposed to LPS showed an attenuated febrile response to LPS exposure in adulthood ($p < .05$). Serum corticosterone levels measured in adulthood were significantly higher in female rats compared to male rats ($p < .05$). Following LPS administration in adulthood, male, but not female, rats neonatally exposed to LPS exhibited lower hypothalamic IL-1β compared to neonatal saline controls. **Conclusion:** The results suggest that neuroendocrine and neuroimmune factors may be correlated with the attenuation of febrile response, that early-life environment is important in determining later-life immune function, and that this effect is sexually dimorphic.

POS-THU-020

NEONATAL LIPOLYSACCHARIDE EXPOSURE IMPAIRS REPRODUCTIVE SUCCESS IN THE RODENT

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Purpose: HPA axis perturbations following perinatal stress, infection or trauma have been well documented. However, despite the central regulatory influence of this system on the HPG axis, the question of whether perinatal events affect reproductive success remains largely unexplored. We investigated the impact of neonatal lipopolysaccharide (LPS) exposure on reproductive development and behaviour in the Wistar rat. Methods: Animals were administered LPS (Salmonella enteritidis, 0.05mg/kg, i.p.; n = 38) or saline (equivolume, ip; n = 37) on days 3 and 5 of life. Daily monitoring from weaning was conducted for markers of puberty (vaginal opening for females, and preputial separation for males), and blood was collected across adolescence to assess testosterone and lutropin levels in males and females, respectively. In adulthood, reproductive behaviour was investigated following adult "stress" (restraint and isolation) or "no stress" (n > 8, for all) given that the long-term effects of neonatal infection have been demonstrated to be amplified by a subsequent stressor in adulthood. All animals underwent sexual behaviour testing with an untreated counterpart of the opposite sex. Blood was collected before and after behavioural testing for corticosterone analysis. Results: Both sexes displayed disruption to the normal weight-to-age ratio of pubertal onset when exposed to neonatal LPS but not saline. Trends indicated increases in lutropin and testosterone levels for LPS-treated animals compared to controls. Neonatal LPS but not adult stress led to impaired adulthood sexual performance indicated by increased mount latencies, fewer mounts and increased rejection behaviour ($p < .05$ for all) for females only. Only animals exposed to neonatal LPS combined with adult stress exhibited significantly higher corticosterone responses ($p < .05$). Conclusion: The current data indicate strong influences of neonatal immune stimulation on later-life parameters of HPA and HPG functioning as well as reproductive development and success, suggesting potential developmental origins for reproductive dysfunction.

POS-WED-021

LOCALIZATION OF THE MALE-SPECIFIC GENE SRY IN THE RAT CENTRAL NERVOUS SYSTEM

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Male and female brains are different in important ways - not just in generating distinct sexual behaviours, but also in terms of memory, emotions, and susceptibility to neurological disorders. Recent studies suggest that sexually dimorphic genes in the adult brain, such as SRY, have an important role in these gender differences. SRY (Sex-determining Region on the Y chromosome) is a male sex-determination transcription factor that directs embryonic gonads to develop as testes, rather than ovaries. Interestingly, SRY plays an important role not just in the male genital development, but also in positively regulating the biochemical and behavioural functions of dopamine neurons in the male substantia nigra (Dewing et al. 2006). The aim of the current study was to examine, by immunohistochemistry, the expression of SRY in the rat brain. To briefly summarise, male rats (n = 3) were anesthetized, transcardially perfused and coronal sections (10µm) of the brains were cut onto slides. The sections were incubated with SRY rabbit polyclonal antibody (generated by Harley laboratory) and visualised by incubation with AlexaFluor488 secondary antibody. SRY immunoreactivity was specific for male rats, with its absence in female rats. SRY immunoreactivity was observed in brain regions involved in voluntary movement (substantia nigra), reward and motivation, (ventral tegmental area), sexual behaviour (medial mammillary body), and spatial memory (dentate gyrus). In the substantia nigra, SRY was found exclusively in tyrosine hydroxylase (TH) neurons. The results from the current study suggest that the male-specific gene SRY is widely distributed in the brain and may be important in regulating various physiological functions (i.e. movement, reward, sexual behaviour) in the male brain.

POS-THU-022

ELUCIDATING SIGNALLING PATHWAYS REGULATED BY UNPROCESSED BDNF

Willingham M.M.[1], Wong A.[1], Junhua X.[1], Perreau V.[1], Kilpatrick T.[1, 2] and Murray S.[1, 2]
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Melanie Willingham1, Agnes Wong1, Junhua Xiao1, Trevor Kilpatrick1,2, Victoria Perreau1, Simon Murray1,2 1Centre for Neuroscience, The University of Melbourne, Victoria, Australia. 2Howard Florey Institute, The University of Melbourne, Victoria, Australia. The neurotrophin Nerve growth factor (NGF) has been found to exert contrasting influences on Schwann cells. NGF, signalling through the p75 neurotrophin receptor (p75NTR), has been found to activate NFκB, which is an essential step for Schwann cell myelination, but also mediate Schwann cell death after peripheral nerve injury. The recent discovery that the unprocessed precursor forms of the neurotrophins (proneurotrophins) are biologically active and promote cell death by acting via a novel signalling complex comprising of p75NTR and Sortilin has raised questions regarding the relative effects of the proneurotrophins and of the mature cleaved protein. Here we identify that p75NTR and Sortilin are expressed in Schwann cells and Dorsal Root Ganglia (DRG) neurons. We show that proNGF and proBDNF have no significant effect on Schwann cell proliferation or differentiation in vitro (n=4), and utilising NFκB luciferase assays show that neither proNGF or proBDNF significantly increase NFκB activation over that seen with the mature forms (n=4). Taken together, these data suggest that proNGF and proBDNF do not play a role in promoting Schwann cell myelination, and that processing of the proneurotrophins is required for these effects. We are currently utilising in vitro myelination assays to further address this question. However, utilising viability assays on Schwann cells, we have established that both proNGF and proBDNF induce significant death in a concentration and time-dependent manner (n=3). Using microarray technology we have identified several key downstream signalling pathways differentially regulated by proBDNF in Schwann cells. We are currently utilising a variety of techniques to validate these changes and elucidate the mechanisms involved in proBDNF-mediated signalling.

POS-WED-023

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND TRKB IN THE PIGLET BRAINSTEM AFTER POSTNATAL NICOTINE AND INTERMITTENT HYPERCAPNIC HYPOXIA

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Purpose: Brain-derived neurotrophic Factor (BDNF) and its receptor TrkB play a significant role in the regulation of cell growth, survival and death during central nervous system development. The expression of BDNF and TrkB is affected by noxious insults. Two insults during the early postnatal period that are of interest to our laboratory are exposure to nicotine and to intermittent hypercapnic hypoxia (IHH). These exposures mimic the conditions associated with the risk factors for the sudden infant death syndrome (SIDS) including postnatal cigarette smoke exposure (nicotine) and prone sleeping where the infant is subjected to re-breathing of expired gases (IHH). Using young developing piglets, we aimed to determine the effects of nicotine and IHH, alone or in combination, on pro- and rh-BDNF and TrkB expression in the brainstem. Methods: Four piglet groups were studied: control (n=14), nicotine (n=14), IHH (n=10) and nic+IHH (n=14). Applying immunohistochemistry, six nuclei of the caudal medulla were studied. Results: Compared to controls, TrkB was significantly decreased after nicotine and nic+IHH exposure regardless of gender. For pro-BDNF and rhBDNF, changes were more evident in males than females exposed to nicotine and nic+IHH. Conclusion: The implications of these findings are that a prior nicotine exposure makes the developing brainstem susceptible to greater changes in the neurotrophic effects of BDNF and its receptor TrkB in the face of a hypoxic insult, and that the effects are greater in males than females.

POS-THU-024

$GABA_A$ RECEPTOR SUBUNIT PROTEIN EXPRESSION IN PREMATURE IUGR PIGLET BRAIN

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Intrauterine growth restriction (IUGR) increases the incidence of various morbidities including prematurity. IUGR can result from prenatal protein malnutrition which is a significant problem in developing countries. Protein malnutrition has been shown to alter mRNA expression of $GABA_A$ receptor subunits in adult rat hippocampus. **Purpose**: To investigate the protein expression of $GABA_A$ receptor α_1 and α_3 subunits in cortex and hippocampus of premature IUGR or normally grown (NG) piglets. **Method**: Premature piglets were obtained by caesarean section at 10 and 14 days prior to term (P-14, NG n = 3, IUGR n = 7; P-10, NG n = 4, IUGR n = 4, respectively). Protein expression levels of $GABA_A$ receptor α_1 and α_3 were analysed using Western blot. **Results**: Overall α_3 expression relative to α_1 was higher in all animals and brain regions across time; there was significantly greater α_3 expression in P-14 IUGR cortex ($p < 0.01$) and P-10 NG hippocampus ($p < 0.001$). Significantly less α_1 was expressed in the hippocampus compared to the cortex of NG animals at P-14 with a similar trend for α_3 in hippocampus. There was a significant difference in α_1:α_3 in the hippocampus of IUGR compared to NG animals ($p < 0.01$). **Conclusions**: Whilst $GABA_A$ receptor α_3 is more highly expressed compared to α_1 in cortex and hippocampus in premature piglets, there appears to be less α_3-containing $GABA_A$ receptors in hippocampus of P-10 IUGR animals. Such differences may contribute to greater vulnerability to brain injury.

POS-WED-025

ATTENUATED RESPONSE OF EPHA4 KNOCKOUT MICE TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Purpose: Multiple sclerosis (MS) is primarily an inflammatory demyelinating disease, however in MS mouse models such as experimental autoimmune encephalomyelitis (EAE) axonal degeneration and reactive gliosis are prominent clinical features. Since astrocytic gliosis is substantially diminished in injured EphA4-/- mice and EphA4 expression inhibits axonal growth, we investigated the clinical course, as well as pathological and histological features of EAE in EphA4-/- mice. **Methods:** EAE was induced by MOG peptide immunisation in C57Bl/6 EphA4-/- mice (n=10) and control wildtype littermates (n=10). Their clinical course was measured daily for 19 days using a functional grading scale. The animals were perfused and their spinal cord sections analysed for inflammatory infiltrates (DAPI and microglial markers CD11b and IBA1) and astrocyte reactivity (GFAP). Axonal damage was analysed using an ELISA to quantitate levels of phospho-neurofilament in their serum. **Results:** Onset of clinical signs of the disease commenced similarly for both cohorts but the disease was more severe in wildtype mice, which reached an average clinical grade of 2.3±0.3 (maximum grade = 4; 60% maximally reached grade 2.5 or higher), while EphA4-/- mice reached an average of 1.7±0.2 (maximum grade 2.75; 60% maximally reached grade 1.5 or lower). Histologically, there were no significant differences in the number of EAE lesions per section, or the number of DAPI nuclei in the lateral white matter (inflammatory infiltrate). However, EphA4-/- mice showed a trend to decreased levels of phospho-neurofilament. **Conclusion:** The clinical course of EAE appears to be less severe in EphA4-/- mice, which may be due to decreased axonal damage, rather than differences in an inflammatory response.

POS-THU-026

HIPPOCAMPAL MOSSY FIBRES EXPRESS RECEPTORS FOR THE COMPLEMENT FACTOR C5A

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Purpose: The complement factor C5a is a major effector of all complement activation pathways. C5a is also produced locally in the CNS along with its receptor, C5aR. This study examined the localization of C5aRs in the developing mouse hippocampus. Methods: The brains from postnatal (P) mice at 4, 8, 13 and 30 days were fixed and processed for immuno-histochemistry (n=3/age). Antibodies to C5aR, as well as neuronal and glial specific antibodies were used on hippocampal sections from these mice. Results: In the adult (P30), intense C5aR immuno-labelling was observed throughout at all rostro-caudal levels of the stratum lucidim within the CA3 region of the hippocampus. Immunolabelling for this receptor did not co-localise with either astrocytic or microgial markers (GFAP and Iba-1 respectively). By contrast, C5aR labelling co-localised with pre-synaptic neuronal markers synaptophysin and synapsin-I, indicating that C5aRs are concentrated at the terminal ends of hippocampal mossy fibres. We next examined the level of C5aR expression at a number of time points during the development of hippocampal mossy fibres (P4, 8, 13). C5aR localization at P13 was similar to that seen in the adult. However at earlier ages (P8, P4), lower levels of C5aR immuno-labeling was observed with a reduced co-localisation to these pre-synaptic markers. Interestingly, additional C5aR immuno-labeling was observed within cell bodies in the dentate gyrus, at P4. Conclusion: Our results show that C5aRs are localized to developing synapses in the stratum lucidim within the CA3 region of the mouse hippocampus. This suggests that C5aRs may have a novel role in synaptic formation and function in this region.

POS-WED-027

AGEING ACCELERATES GLUTATHIONE EXPORT FROM CULTURED ASTROCYTES

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Purpose: Perturbations of glutathione trafficking between astrocytes and neurons have been implicated in the neurological dysfunction that accompanies ageing and age-related neurodegenerative conditions. Astrocytes are responsible for synthesising and exporting glutathione to provide glutathione precursors to neurons, thus we have investigated whether ageing alters the capacity of the astrocytic glutathione system. **Methods:** Astrocytes were cultured from the brains of neonatal (<24h), adult (12-month-old), and aged (24-month-old) C57BL/6J mice. Glutathione content was measured in the 24h following application of the glutathione synthesis inhibitor, buthionine sulfoximine (BSO). Additionally, the rate of glutathione export into the media was determined over the first 4h. Experiments were performed on at least three independent cultures for each age group. **Results:** BSO treatment depleted neonatal cultures of glutathione with a halftime of 3.7h. In contrast, glutathione was depleted significantly faster from adult and aged cultures, with halftimes of 2.3h and 2.6h respectively. There was a linear increase in extracellular glutathione for all cultures, with neonatal astrocytes exporting glutathione at a rate of 3.3% of total glutathione/h. In contrast, adult and aged cultures exported glutathione 2.3 and 2-fold faster than neonatal astrocytes, respectively. In the presence of BSO, an additional 7% of total glutathione/h was lost from all cultures. **Conclusions:** While there were no age-related differences in the cellular consumption of glutathione, astrocytes cultured from adult and aged mice exported glutathione much more rapidly, causing faster depletion of this antioxidant. Accelerated glutathione export may support a greater demand for glutathione by neurons in the aged brain, however this may render astrocytes vulnerable to pro-oxidants, reducing their capacity to support neuronal function.

POS-THU-028

REGULATION OF ASTROCYTE ACTIVATION AND FOCAL ADHESION FORMATION BY RHO-KINASE AND EPHA4 IN CULTURED ASTROCYTES

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Purpose: Spinal cord injury in EphA4 null mice revealed functional recovery compared to wildtype animals. This appeared to involve a lack of robust astrogliosis, with only a modest increase in GFAP expression in EphA4 null animals (Goldshmit et al., J. Neurosci. 2004). **Methods and Results:** To compare cytoskeletal protein expression, proliferation and migration in wildtype and EphA4 null astrocytes in vitro, we conducted scratch wound assays as a model of astrocyte activation. GFAP expression in astrocytes invading the scratched area and in control areas 250µm next to the scratch was assessed. There were significant differences between cells that migrated into the scratch and cells 250µm next to the scratched area. At all time points there were significantly less EphA4 null astrocytes that migrated into the scratched area that were GFAP positive in comparison to control areas. Immunocytochemistry for Vimentin revealed an increase in expression. No differences in Vimentin expression between genotypes in cells invading the scratch were detected. At all time points astrocytes of both genotypes invading the scratch were all found to be F-actin positive. Investigation of scratch wounds after Rho-kinase inhibition (25µM HA1077) led to increased proliferation/migration in wildtype astrocytes. GFAP expression was increased after 24h in EphA4 null scratched astrocytes, indicating a role of Rho-kinase in astrocyte activation. Eph signaling also regulated astrocyte adhesion and focal adhesion formation. EphA4 null astrocytes showed decreased adhesion while ephrinA5-Fc induced cells of both genotypes to increase vinculin positive focal adhesion numbers and size. **Conclusion:** Eph signaling regulated a number of astrocyte cytoskeletal responses in culture.

POS-WED-029

INVESTIGATION ON THE ROLE OF OLIGODENDROCYTE-EXPRESSED TRKB IN CENTRAL NERVOUS SYSTEM MYELINATION

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Purpose: Myelination requires a highly organised and complex array of cellular interactions between neurons and oligodendrocytes in the Central Nervous System (CNS). The mechanisms required to achieve this process are yet to be fully elucidated. Our laboratory is currently investigating the role of Brain Derived Neurotrophic Factor (BDNF) in regulating oligodendrocyte myelination. **Methods & Results:** Using the *in vitro* myelination assay, where the co-culture of dorsal root ganglia (DRG) neurons with oligodendrocyte precursor cells (OPCs) replicates the myelination process, our data indicate that BDNF promotes oligodendrocyte myelination *in vitro* (n=3). BDNF signals through two types of receptors: the TrkB tyrosine kinase receptor and the structurally unrelated p75 receptor. Using RT-PCR, we could detect p75 expression in both DRG neurons and OPCs, however TrkB FL was restricted to the cells of oligodendrocyte lineage (n=3). Addition of K252a, a tyrosine kinase inhibitor, into DRG-OPC coculture significantly reduced BDNF enhanced myelination (n=3), suggesting that TrkB signaling mediates the increased myelination by BDNF. **Conclusion:** With the restricted expression profile of TrkB on OPCs, these data suggest that oligodendrocyte-expressed TrkB receptors promote oligodendrocyte myelination. We are currently testing this by a genetic approach utilising TrkB $^{fl/fl}$ mice, and conditionally deleting TrkB from oligodendrocytes. We are currently investigating CNS myelination in this mouse model.

POS-THU-030

P2X4 RECEPTORS ARE ASSOCIATED WITH ACTIVATED MICROGLIA IN THE IMMATURE RAT BRAIN AFTER HYPOXIA-ISCHEMIA

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Purpose: Neuroinflammation, partly defined by a robust increase in the number of activated microglia, is a key secondary injury process that contributes to white matter damage in preterm neonates exposed to hypoxia-ischemia (HI). However the mechanisms by which microglia signal in the injured brain are not clear. Methods: Using a preterm HI model (right common carotid artery + exposure to 6% O_2) in the post-natal day 3 (P3) rat, we investigated whether the expression of purine ionotropic $P2X_4$ receptors ($P2X_4R$) change in the brain after P3 HI. In control (n=31) and HI animals (n=33) we determined the levels of $P2X_4R$ and Iba-1 (microglial marker) protein expression from P4-P10 and identified the cellular localisation of $P2X_4R$ after P3 HI. We also examined whether blocking HI-induced activated microglia (minocycline 45 mg/kg; n=9) altered the expression of $P2X_4R$. Results: $P2X_4R$ expression increased after P3 HI ipsilateral to the carotid ligation. Dense $P2X_4R$-immunoreactivity was evident in the corpus callosum and primarily co-localised on activated microglia. In the cingulum, immediately dorsal to the corpus callosum, co-localisation with neurons was also apparent. Minocycline treatment attenuated the HI-induced increase in ipsilateral $P2X_4R$ expression and reduced $P2X_4R$ immunolabelling in the corpus callosum but not the cingulum. However, $P2X_4R$ expression was only associated with a second, late rise in Iba-1 expression from P8 indicative of microglia cell activation. Conclusion: We postulate that $P2X_4R$-positive microglia may represent a distinct population of secondary injury-induced activated microglia and may contribute to the progression of injury in the HI-affected immature brain.

POS-WED-031

IS THE FUNCTION OF P75 REGULATED BY ITS CELLULAR LOCALISATION MEDIATED BY SORTILIN INTERACTIONS?

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The p75 neurotrophin receptor is a type I transmembrane receptor for the neurotrophin family of ligands. p75 can signal to initiate apoptosis, and it has been suggested that the p75 co-receptor sortilin is necessary for death signalling. We have evidence that cell death signalling is initiated following cleavage removing the extracellular domain and hypothesised that increased levels of p75 in the plasma membrane and/or in recycling endosomes following cleavage would contribute to cell death signaling. To test this hypothesis we generated a series of p75 mutants fused to fluorescent proteins for assessment in cell culture systems by confocal microscopy. Firstly the roles of the co-receptors sortilin and survival-promoting TrkA on p75 subcellular redistribution were tested. Sortilin co-expression increased the level of p75 at the cell surface, compared to that of TrkA or control protein ß-galactosidase co-expression. Secondly, the location of p75NTR under conditions whereby cleavage was mimicked or stimulated was assessed. Constructs lacking the extracellular domain but retaining the signal peptide, transmembrane and intracellular domains, were not present at discernable levels in the plasma membrane. By contrast, constructs that expressed a minimal extracellular and intracellular domains as well as signal peptide and transmembrane domains expressed well at the plasma membrane. These results suggest that one or more factors could bind the extracellular domain to facilitate p75 trafficking to the plasma membrane and/or bind the intracellular domain to retain p75 in the Golgi/endoplasmic reticulum. The ability of sortilin to regulate the cellular location of these and other truncated p75 proteins is now being investigated. Our finding may explain the necessity of sortilin expression for p75 to mediate cell death.

POS-THU-032

CORTICOTECTAL PROJECTIONS IN EPHRIN-A-/- MICE

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Purpose: Topographically ordered projections from the retina and primary visual cortex (V1) converge on the superficial layers of the superior colliculus (SC). Retinotectal topography is established by the graded expression of various molecules in the retina and SC, in particular ephrin-A2 and ephrin-A5 ligands in the SC and EphA receptors in the retina. Studies in knockout mice show that retinotectal topography is more disordered in mice lacking both ephrin-A2 and -A5 (89%) compared to mice lacking only ephrin-A2 (57%). In both knockout (-/-) genotypes, ectopic retinotectal terminations are found. By contrast, the pattern of retina-to-dorsal lateral geniculate nucleus (dLGN), and dLGN-to-V1 projections in these mice resembles that seen in normal animals. Here we analyze the projection from V1 to SC in ephrin-A-/- mice. **Methods**: Two fluorescent tracers (BDA, green or red) (150-300nL) were injected into V1 of wild-type (n=7), ephrin-A2/A5-/- (n=5) or ephrin-A2-/- (n=10) mice. We examined m coronalµthe number, location and size of anterograde termination zones in 40 sections of the SC, and mapped the distribution of retrgradely labelled neurons in dLGN. **Results**: There was no difference in injection size or number of labelled cells in dLGN between genotypes or based on the location of the injection. Nonetheless, abnormal corticotectal projections were observed in all ephrin-A2/A5-/- mice and in 10% of ephrin-A2-/- mice. There were no abnormal termination zones in wild-type mice. Furthermore, when compared to wild-type and ephrin-A2-/- mice the volume of termination zones in the SC was smaller in ephrin-A2/A5-/- mice (p=0.002). **Conclusion**: Our results suggest that the development of the corticotectal topographic map is guided by the previously established retinotectal map in the superficial SC.

POS-WED-033

WNT SIGNALING IN DOPAMINERGIC AXON GUIDANCE

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Purpose: An ongoing challenge for biologists is to understand the intricate and precise pattern of connectivity achieved during brain development. Whilst many guidance molecules have been identified within the midbrain dopamine (DA) pathways, they fail to account for all events. Elsewhere within the central nervous system Wnts have been identified in axon guidance/synaptogenesis. Interestingly, Wnts have also been shown to be important in the proliferation and differentiation of DA neurons in development. We endeavoured to establish whether Wnts also play a role in DA axon morphogenesis in the developing brain. **Methods:** 1.Examine the temporal and spatial expression of Wnts during midbrain DA development using in situ hybridisation. 2. Study the role of Wnts in axon morphogenesis examining (i) neurotopism in midbrain primary cultures (during periods of DA neurite outgrowth, E11.5, and neurite elongation, E14.5), including antagonism (n=5) and, (ii) neurotrophism in explant co-cultures (n=12). **Results:** Wnt1, Wnt3a, Wnt5a and Wnt7a showed distinctive temporal and spatial expression in the developing DA pathways, suggestive of roles in DA neurite morphogenesis. At E11.5, DA primary cultures showed these Wnts promoted neurite extension (up to 3-fold for Wnt3a and 5a, $p>0.005$), effects that were reduced or reversed by E14.5. Wnt related antagonists confirmed that these effects were mediated through both canonical and non-canonical Wnt pathways. Furthermore, we showed Wnts1, 3a, and 7a induced chemoattractive effects for DA neurites while Wnt5a was chemorepulsive. **Conclusions:** We hereby demonstrate key roles for Wnts in (i) initiation of DA neurite outgrowth (ii) neurite elongation and, (iii) DA chemotaxis. These findings increase our understanding of how midbrain DA pathway are wired, and provide new avenues to regulate DA axon guidance in cell replacement therapy for Parkinson's disease.

POS-THU-034

ANTI-MUSK-POSITIVE MYASTHENIA GRAVIS PATIENT ANTIBODY CAUSES ABERRANT ACTIVATION OF MUSK AND DISASSEMBLY OF ACETYLCHOLINE RECEPTOR CLUSTERS

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Purpose: Myasthenia gravis (MG) is an antibody-mediated autoimmune disease of the neuromuscular junction (NMJ) that causes impaired neuromuscular transmission and muscle weakness. Muscle Specific Kinase (MuSK) is recognized as the auto-antigen in about 10% of MG patients. Activation of MuSK by neural agrin is essential for acetylcholine receptor (AChR) clustering at the NMJ. Here we aimed to define the mechanisms by which anti-MuSK antibodies interfere with the postsynaptic AChR cluster. **Methods**: To investigate the effect of anti-MuSK antibodies on pre-existing AChR clusters, cultured mouse C2 myotubes were pre-treated with agrin (1nM, 4h) to form AChR clusters and then exposed to control human IgG or IgG from an anti-MuSK-positive MG patient. Confocal optical sections of control and experimental myotubes were used to compare the number and size of AChR clusters. Immunoprecipitation followed by immunoblotting was used to assess the effect of anti-MuSK antibodies on tyrosine phosphorylation of MuSK. To assess the effect of anti-MuSK antibodies on the subcellular distribution of MuSK, C2 myoblasts transfected with MuSK-GFP were incubated with control IgG or patient anti-MuSK IgG and then immunofluorescently stained with Cy3-anti-human IgG. Myoblasts transfected with MuSK-GFP were also imaged live on a confocal microscope. **Results**: Myotubes exposed to anti-MuSK antibodies displayed a significant reduction in number and size of AChR clusters compared with cells treated with control human IgG (n=3). Anti-MuSK antibodies caused cross-linking, auto-phosphorylation and internalization of MuSK (n=3). **Conclusion**: Anti-MuSK auto-antibodies cross-link and activate MuSK. This somehow causes the disassembly of large pre-existing AChR clusters.

POS-WED-035

LTP ACTIVATES LATENT PRECURSOR CELLS IN THE ADULT MOUSE DENTATE GYRUS

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(Purpose) Since we recently demonstrated neural activity can stimulate hippocampal precursors in vitro (1), we examined whether long term potentiation (LTP) enhances proliferation of the neural precursors and neurogenesis in the dentate gyrus of adult mice. (Methods) We stimulated the perforant pathway unilaterally and LTP was induced by high frequency stimulation (HFS). In addition, some mice were given low frequency stimulation (LFS) unilaterally. The unstimulated side was regarded as the control. Mice were administrated BrdU intraperitoneally four days after stimulation and then sacrificed 30 minutes later and analyzed for proliferation of the neural progenitor cells. Also, some mice were assayed for neurosphere formation two days after stimulation. (Results) Hippocampus stimulated by HFS had a significantly higher number of BrdU positive cells than the control side, whereas mice given LFS didn't have a statistically significant change in the number of BrdU positive cells between LFS side and the control side. We found also an increase in the number of neurospheres in hippocampus with LTP stimulation compared to the LFS and unstimulated sides. (Conclusion) LTP activates latent neural precursor cells in the adult mouse dentate gyrus leading to increased neurogenesis.
(1) Walker, T. L., A. White, et al. (2008). "Latent stem and progenitor cells in the hippocampus are activated by neural excitation." J Neurosci 28(20): 5240-7.

POS-THU-036

ENDOGENOUS IFNγ DIRECTLY REGULATES NEURAL PRECURSORS IN THE NON-INFLAMMATORY BRAIN

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Purpose: To examine the role of interferon-gamma (IFNγ) in the regulation of neural precursor (NP) activity in the subventricular zone (SVZ) of adult mouse brain. **Methods**: Neurosphere assay and Brdu intrapertoneal injection were used. **Results**: We found IFNγ and its receptor are highly expressed in the SVZ. Exogenous IFNγ treatment produces a marked decrease in neurosphere formation, with an approximately 50% drop in neurosphere frequency observed in the presence of IFNγ. We confirmed that the reduction is not due to the increased cell death, as demonstrated by a similar reduction in neurosphere formation with IFNγ treatment in the Bax mutant mice to that in wild-type (WT) control. The in vitro findings was confirmed by the in vivo observation that the number of Brdu-positive cells from adult IFNγ-/- SVZ was 2-fold greater than that from control SVZ. In addition, more neurospheres were formed from the IFNγ-/- SVZ than that from WT controls. Furthermore, cell sorting for the IFNγ-responsive cells, using expression of major histocompatibility complex class I (MHCI) as a marker of IFNγ-mediated activation, revealed that nearly all the neurospheres were derived from the MHCI-positive population, indicating that IFNγ acts directly on NPs. However, the inhibitory effect of IFNγ was modulated by microglia, as their removal resulted in almost complete inhibition of NP proliferation (90% versus 50% for unsorted cells). Thus all the precursors are susceptible to IFNγ, with microglia able to protect a subpopulation of the neurosphere-forming cells from its inhibitory effects. **Conclusion**: these findings demonstrate that, in the normal brain, IFNγ acts as a regulator of the active NP pool, an effect which is modulated by microglia.

POS-WED-037

THE EFFECTS OF METALLOTHIONEIN-IIA ON WOUND HEALING AND NOCICEPTIVE FUNCTION FOLLOWING BURN INJURY

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Purpose: Following burn injuries, rapid healing is associated with reduced scarring. However, despite reduced scarring, severe sensory deficits often persist clinically. We recently showed that a single application of metallothionein II-A (MT-IIA), a naturally occurring cysteine-rich protein, enhances HaCaT cell viability and migration compared to saline controls ($p<0.05$) *in vitro*, and accelerates healing and reduces scarring ($p<0.05$) following burn injury *in vivo* in mice. Here we determined whether MT-IIA also improved sensory function after burn injury. **Methods:** Nociceptive function was measured weekly for eight weeks following a burn injury to the mid-back region in mice. Mechanical stimuli (von Frey filaments, 15-300g) were applied to the injury site and at two equidistant locations rostrally and one caudally thus encompassing the dorsal rostro-caudal midline. Nociceptive function was examined over 8 weeks by 2 independent observers rating cutaneus trunci muscle reflex vigour; data were averaged to give a graded response score. **Results:** Compared to normal animals (unburned), there was a significant reduction in graded responses at all locations near and distant to the burn wound for up to eight weeks following a burn injury; reductions occurred regardless of treatment (saline, zinc and MT-IIA treated animals, $p<0.05$). **Conclusions:** Although MT-IIA reduces scarring following burn injury, other treatments such as sensory retraining may be necessary to restore normal nociception at the injury site as well as maintain it in intact skin at distant locations.

POS-THU-038

SOCS2 REGULATES NEURITE OUTGROWTH AND NEURONAL SIGNALLING THROUGH TRK RECEPTOR INTERACTIONS

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Purpose: SOCS2 can regulate neurite outgrowth in PC12 cells and cortical neurons. Overexpression of SOCS2 promoted an increase in neurite length and neurite number (Goldshmit 2004). The mechanism by which SOCS2 regulates these processes is unresolved. **Methods:** Wildtype or mutant SOCS2 proteins, or a GFP-only control were expressed in PC12 cells and neurite outgrowth examined under basal proliferative conditions and with Nerve Growth Factor (NGF) which promotes neuronal differentiation. The Flag-tagged SOCS2 mutant expression constructs included deletion of the N terminus (delta NT), deletion of the SOCS box (delta Box), or specific point mutations of conserved residues within the SOCS2 SH2 domain (R73K (SH2 1KD) or R73K/D74E/S75C (SH2 3KD)). As NGF signals through the TrkA receptor, levels of TrkA expression were analysed in PC12 cells transfected with wildtype or mutant SOCS2 proteins and the phosphorylation of p42/44 MAP kinase was examined upon NGF stimulation. **Results:** Full length SOCS2 was required to promote neurite outgrowth. Although the N terminal domain of SOCS2 was partially dispensable, an intact SH2 domain and SOCS box domain were both required for promotion of neurite outgrowth. Deletion of the SOCS box prevented neurite outgrowth under basal conditions and blocked NGF-induced neurite outgrowth, thus behaving as a potent dominant negative mutation. Increased total TrkA levels correlated with increased neurite outgrowth. Map kinase phosphorylation was more intense and prolonged with wildtype SOCS2 than under control conditions and less intense and for a shorter time with the delta Box mutant. **Conclusion:** SOCS2 and SOCS2 mutants can modulate expression of the TrkA receptor and regulate the intensity and length of MAPK signalling downstream of NGF stimulation in PC12 cells.

POS-WED-039

ONTOGENY OF VITAMIN-D RECEPTOR EXPRESSION IN RAT MESENCEPHALON

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Purpose: Developmental Vitamin-D (DVD) deficiency is a candidate risk factor for schizophrenia. Transient vitamin-D deficiency in pregnant rats has been shown to alter neurodevelopment and behaviour in offspring. Both cellular and behavioural findings have strongly implicated alterations in the ontogeny of dopamine signalling in DVD-deficient offspring. This study aimed to investigate the ontogeny of the vitamin-D receptor (VDR) in developing Tyrosine-hydroxylase (TH)-positive (dopaminergic) neurons. **Methods:** Control Sprague-Dawley rats at day 12 (E12) or 15 (E15) of gestation and postnatal days 0 (P0), 21 (P21) and 70 (P70) were euthanased and their brains perfused and fixed for immunofluorescence. Midbrain regions were cut at 20 µm (postnatal) or 50µm (embryonic) and incubated with primary antibodies against VDR or TH, which were visualised with fluorescent-conjugated secondary antibodies Alexa-488 or Cy3, respectively. Imaging was performed on a Zeiss AxioImager fluorescent microscope with ApoTome optical sectioning. **Results:** There was only sparse VDR immunoreactivity at E12 despite the presence of TH-positive cells. All TH-positive cells expressed VDR from E15 to P70. VDR labelling was predominantly nuclear, but became more somal with increasing age. VDR staining was also present in non-midbrain sections of all ages. **Conclusion:** From E15 to adulthood, dopaminergic neurons were seen to express a nuclear vitamin-D receptor, indicating that vitamin-D could play some role in dopamine ontogeny.

POS-THU-040

DEVELOPMENTAL VITAMIN D (DVD) DEFICIENCY ALTERS MK-801 INDUCED LOCOMOTION AND GLUTAMATERGIC RECEPTOR BINDING

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Purpose: Developmental vitamin D (DVD) deficiency has been proposed as a risk factor for schizophrenia. We have shown that DVD-deficiency in the rat results in an enhanced locomotor response to MK-801 in young adult male rats, which can be ameliorated with haloperidol. The aim of this study was to examine the effects of DVD-deficiency in 6-month old rats on locomotor response to MK-801 and binding for dopaminergic and glutamatergic receptors. **Methods:** Female Sprague-Dawley rats were fed a vitamin D deficient diet for 6 weeks prior to conception and maintained on this diet until birth. Control dams were fed a vitamin D normal diet. The offspring of these dams were reared on a diet containing vitamin D and tested at 6 months of age for locomotion in an open field or used for radioisotope binding to quantify neurotransmitter receptor densities. **Results:** Both male and female DVD-deficient rats showed enhanced MK-801 induced locomotion compared to controls ($P<0.05$). Overall, DVD-deficient rats also showed a decrease in MK-801 binding in the striatum ($P<0.05$) but no change in receptor binding for dopamine. **Conclusion:** DVD-deficiency alters behaviours and receptor binding with relevance to glutamatergic neurotransmission. We suggest that a decrease in NMDA receptors in the striatum may be exacerbated by MK-801 induced antagonism, which leads to an increased glutamatergic overflow resulting in the increased activation of alternative glutamatergic receptors i.e. AMPA/kainate. The results shown here indicate that DVD-deficiency impacts on the development of glutamatergic systems in the rat.

POS-WED-041

DEVELOPMENTAL VITAMIN D (DVD) DEFICIENCY ALTERS BRAIN ANATOMY IN THE ADULT MOUSE

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Introduction: Developmental vitamin D (DVD) deficiency has been proposed as a risk factor for several brain disorders. DVD-deficiency alters brain development and behaviour in rats, and behaviour in mice. The aim of this study was to investigate the role of vitamin D in brain development by examining the effect of DVD-deficiency on neuroanatomy in two strains mice. **Methods:** Female C57BL/6J and 129/SvJ mice were fed a vitamin D-deficient diet from 6-weeks prior to conception until birth, and subsequently transferred to a diet containing vitamin D. Control dams were fed a vitamin D normal diet throughout the experiment. Brains of C57BL/6J and 129/SvJ neonatal (P0) and adult (P70) mice were imaged using magnetic resonance imaging and the volumes of the brain, hippocampus, striatum, septum, cortex and ventricles measured, as well as the widths of white matter tracts. All internal brain structures were corrected for total brain volume. **Results:** DVD-deficiency had no effect on neonatal mouse brain anatomy. In adult male mice, there was a significant decrease (30%; $P<0.05$) in lateral ventricular volume and an increase (5.5%; $P<0.05$) in striatal volume, which was not seen in females. These measures were negatively correlated with each other in DVD-deficient mice ($r=-0.8$; $P<0.5$). **Conclusions:** This study revealed a subtle but significant change in the neuroanatomy of DVD-deficient mice. The decrease in lateral ventricular size independent of a change in overall brain volume suggests the ventricles were compressed by other structure(s), perhaps the striatum. This data further implicates DVD deficiency as an active agent in brain development.

POS-THU-042

ALTERED INHIBITORY SYNAPTIC TRANSMISSION IN SPASTIC AND SPASTIC-RESCUE MICE

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The spastic mouse has a naturally occurring mutation in the beta-subunit of the glycine receptor (GlyR), which produces a severe motor phenotype. Molecular and behavioural studies have shown insertion of a rat beta-subunit transgene into spastic mice can increase beta-subunit expression levels (from 10 to 25% of normal) and abolish the spastic phenotype (Hartenstein et al., 1996 EMBO J 15:1275). **Purpose**: To compare the electrophysiological properties of native GlyRs in spastic and spastic-rescue mice in adult animals and during postnatal development. **Method**: Mice (C57Bl/6; both sexes) were anaesthetized with Ketamine (100 mg/kg, i.p.) and decapitated. Whole cell recordings (holding potential -70mV) were obtained from hypoglossal motoneurons in brainstem slices at 23oC using a CsCl-based internal. **Results**: Glycinergic mIPSCs were isolated in CNQX (10 μM), TTX (1 μM), bicuculline (10 μM) and blocked by strychnine (1 μM). In adult animals (P27-32) mean mIPSC amplitude was reduced in spastic (~ 30% of control) and spastic-rescue (~ 45% of control) animals compared to wild type mice (21.0 ± 2.1, n = 19 vs. 33.6 ± 3.5, n = 20 vs. 71.7 ± 3.5 pA, n = 23). mIPSC decay time was similar (~ 5 ms) in each genotype. During development (P0 –25) mIPSC amplitude remained constant in spastic (~ 20 pA), increased slightly in spastic-rescue (25.4 ± 3.4 to 33.6 ± 3.5 pA), and markedly in controls (37.3 ± 2.4 to 71.7 ± 3.5 pA). mIPSC decay time constant decreased similarly each genotype (~ 15 to ~ 5 ms). **Conclusion**: These data suggest a critical level of glycine receptor expression (> 30% of normal) is required for appropriate maintenance of motor behaviour.

POS-WED-043

GLYCINE RECEPTOR MEDIATED SYNAPTIC TRANSMISSION IN THE SUPERFICIAL DORSAL HORN OF *SPASMODIC* MICE

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The *spasmodic* mouse has a single point mutation (A52S) in the α1 subunit of the glycine receptor (GlyR). This mutation results in a marked motor phenotype characterised by an exaggerated startle reflex, resting tremor, and impaired righting reflex. We have previously shown that the mutation reduces agonist sensitivity in standard α1/β subunit containing GlyRs and speeds up receptor kinetics in brainstem motoneurons. In the superficial dorsal horn (SDH) of the spinal cord, however, the GlyR α3 subunit is also expressed, potentially preserving some GlyR function in *spasmodic* mice. **Purpose**: To assess the impact of the *spasmodic* mutation on GlyR-mediated synaptic transmission in SDH neurons. **Methods**: Mice (C57Bl/6 and *spasmodic*, 23-42 days) were anaesthetised (Ketamine; 100 mg/kg, i.p.) and decapitated. Transverse spinal cord slices (300 μm) were prepared (L3-L5) and GlyR-mediated miniature inhibitory postsynaptic currents (mIPSCs) were recorded (CsCl internal) in voltage clamp (holding current -70 mV) at room temperature. **Results**: GlyR-mediated mIPSC frequency (0.13 ± 0.03 vs. 0.16 ± 0.02 Hz) and rise time (0.86 ± 0.05 vs. 0.76 ± 0.04 ms) were similar in *spasmodic* and control animals (n=34 & n=33 respectively). Peak mIPSC amplitude was significantly reduced (30.9 ± 1.8 vs. 41.9 ± 3.6 pA), and mIPSC decay time-constants were faster (4.90 ± 0.38 vs. 7.37 ± 0.48 ms) in *spasmodic* animals. The combined effect of these altered characteristics was to significantly reduce charge transfer per mIPSC in *spasmodic* mice (167.7 ± 57.7 pA.ms vs. 354.2 ± 39.9 pA.ms). **Conclusions**: These data indicate that GlyR α3 subunit expression in the SDH is insufficient to preserve normal glycinergic drive in *spasmodic* mice.

POS-THU-044

RESPONSES TO CURRENT INJECTION DIFFER BETWEEN MOUSE CERVICAL, THORACIC AND LUMBAR SUPERFICIAL DORSAL HORN NEURONS

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Superficial dorsal horn (SDH; laminae I-II) neurons are important for pain signaling. Surprisingly, much of what we know about the electrophysiological properties of SDH neurons comes from studies restricted to lumbar segments of the spinal cord. **Purpose**: To compare the intrinsic membrane properties and excitability of SDH neurons from cervical, thoracic and lumbar spinal cord segments. **Method**: Mice (C57Bl/6; both sexes, ~P30) were anaesthetized with Ketamine (100 mg/kg, i.p.) and decapitated. Transverse slices were prepared from cervical (C2-4), thoracic (T8-10) and lumbar (L3-5) spinal cord segments. Whole cell recordings were obtained from SDH neurons at 32°C using a potassium-based internal solution. **Results**: Neuronal input resistance (428 ± 21 vs. 426 ± 23 vs. 420 ± 18 MOhms), capacitance (17.4 ± 0.7 vs. 17.0 ± 0.6 vs. 17.5 ± 0.7 pF), and resting membrane potential (-65.8 ± 1.0 vs. -68.3 ± 1.2 vs. -67.3 ± 1.1 mV) were similar at each spinal level (cervical (n = 99), thoracic (n = 93) and lumbar (n = 102)). Action potential (AP) height, half-width and AHP amplitude were also similar between levels. Neurons were grouped into five AP discharge categories according to their response to current injection. The prevalence of *tonic firers* and *delayed firers* was similar at all levels. At cervical levels, however, *initial bursters* were more prevalent, whereas *single spikers* and *reluctant firers* were less common (Chi-square = 16.69, df = 8, p = 0.03). **Conclusion**: These data suggest different pain processing mechanisms operate in the SDH along the rostro-caudal axis of the spinal cord.

POS-WED-045

PROPERTIES OF THE MEDIAL AMYGDALA NEURONS IN MICE

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BACKGROUND: The medial nucleus of Amygdala (MeA) is a subcortical structure that processes pheromonal signals and plays a key role in regulating social and reproductive behaviour. **METHODS:** To study the electrophysiological properties of MeA neurons, we performed whole-cell recording using acute brain slices from male GAD67 knock-in mice. Both adult (2-4 months) and juvenile (3-4 weeks) mice were used and recordings were made from either GFP+ or GFP- cells. **RESULTS:** A total of 18 cells with a resting membrane potential more negative than -55 mV and access resistance less than 15 MΩ were recorded, among which 9 were from the juvenile and 9 from the adult mice. 55% (5/9) of juvenile and 77% (7/9) of adult cells were GFP+. In response to a depolarising current injection, these cells showed a range of firing properties varying from those with clear spike frequency adaptation to those with repetitive firing throughout the depolarising current step. 44% (4/9) of adult and 77% (7/9) of juvenile cells showed complete accommodation after 1-3 spikes. The rest of the cells fired more than five spikes with fully accommodating (two adult, one juvenile), stuttering (one adult, one juvenile cell), and none-accommodating (one adult) firing patterns. GFP+ and GFP- cells did not differ in their firing properties. A hyperpolarization-activated cation current (Ih) was found in 44% and 77% of the adult and juvenile cells, respectively. We conclude from our recordings that the majority of the MeA cells are accommodating and that there is a good agreement between firing properties of GFP+ and GFP – and between that of adult and juvenile cells.

POS-THU-046

PROPERTIES OF THE INTERCALATED NEURONS IN THE RODENT AMYGDALA

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The intercalated cell masses (ITCs) of the amygdala are a cluster of interneurons, located between the basolateral complex (BLA) and central nucleus, the main input and output stations of the amygdala. These neurons play an important role during extinction of conditioned fear and act as feed forward interneurons for cells in the central amygdala. However, their physiological properties are little understood. **Methods:** In order to characterize these interneurons we performed whole-cell recordings, using acute slices from GAD67-EGFP transgenic mice. **Results:** Three different firing patterns (n=42) were found. 43 % (18/42) of ITCs fired more than one action potential but showed clear adaptation after 1-3 spikes. 11/18 of these cells showed a clear delay in the initiation of the first spike. In 29 % (12/42) neurons showed no spike frequency adaptation during the 700 ms current injection. 10 % (4/42) of ITCs fired with variable spike frequency adaptation. Synaptic properties onto 73 ITCs were studied by stimulating fibres in the BLA. In the presence of picrotoxin (100 µM) a two component excitatory postsynaptic current with I-V relations typical for AMPA receptors and NMDA receptors were observed. Subsequent examination of the receptor subunit composition revealed that these synapses express GluR2-containing and GluR2-lacking AMPA receptors. The NMDA receptor mediated EPSC showed both NR2A and NR2B receptor components at different synapses. In 82% (60/72) of the recordings a large polysynaptic inhibitory response was seen suggesting these cells are interconnected. In further studies we will investigate wether synapses onto the ITCs are plastic and if these cells could not only be a site for the expression of extinction but also a site for the storage of extinction memory.

POS-WED-047

TRAFFICKING OF SK2 CHANNELS

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Purpose: Small conductance calcium-activated potassium channels (SK channels), underlie the medium after-hyperpolarisation (mAHP), which modulates firing frequency in many neurons. SK channels are also located in spines, where they attenuate synaptic potentials following activation by calcium influx through NMDA receptors. This process is regulated by SK channel internalization. Recent research in COS7 cells has shown that activation of PKA by forskolin induces internalization of surface SK2 channels. This project aims to examine trafficking of one type of SK channel (SK2 channels) both *in vitro* and *in vivo*. **Methods:** An SK2 construct containing an extracellular triple myc epitope was cloned into the FCK1.3GW vector, under the control of the CamKII promoter to drive pyramidal neuron specific co-expression with EGFP. Third generation lentiviruses were produced of the FCK (1.3) SK2 IGW vector. Primary neuronal cultures and HEK293T cells were transfected with the SK2 construct and surface expression was studied by immunofluorescence. **Results:** Transfection of the SK2 produced clustered staining in HEK293T cells (n=20) and punctate staining throughout the dendritic trees of primary prefrontal cortical neurons (n=15) and primary hippocampal neurons (n=15). Forskolin (50 µm) decreased surface expression in HEK cells by 35%, and produced similar results in neuronal cultures. In vivo stereotactic injection of the lentiviral constructs into the basolateral amgydala (BLA) of 12 day old Wistar rats yielded immunohistochemically detectable neuronal expression of myc-tagged SK2 and EGFP. **Conclusions:** Trafficking of the SK2-myc construct is modulated by forskolin in cultured HEK293T cells, in cultured primary hippocampal and prefrontal cortex neurons. The SK2 lentiviral vector will be a useful tool for studying trafficking and modulation of SK2 channels *in vivo*.

POS-THU-048

SUBUNIT COMPOSITION OF NMDA RECEPTORS IN THE CENTRAL AND BASOLATERAL AMYGDALA

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Electrophysiological recordings from neurons in the central nucleus of the amygdala (CeA) and the basolateral amygdala (BLA) reveal that NMDA receptors at glutamatergic synapses in the CeA contain NMDA receptors with kinetics and pharmacological properties consistent with a NR2B phenotype whereas those of the BLA were more NR2A-like. Here we begin to examine the subunit composition of NMDA receptors in these nuclei using molecular, RNAi and protein expression techniques, and electrophysiological recordings. **Methods:** Western blots and purification of PSD fractions from CeA and BLA tissue dissected from acute rat brain slices, indicated the expression of both NR2A and NR2B in both the CeA and BLA. We then used short hairpin RNA (shRNA) constructs to specifically knockdown each of these subunits in the CeA and BLA to determine whether native NMDA receptor properties would be maintained in the absence of each subunit type. Organotypic rat brain slices were grown using the Stoppini method and transfections of shRNA constructs were carried out using a Helios Gene Gun (Biorad). **Results:** Down-regulation of NR2A subunits in the BLA resulted in slowing down of the NMDA receptor mediated excitatory post synaptic current (EPSC) and increased ifemprodil sensitivity, whereas knockdown of NR2B subunits in the CeA resulted in faster decaying NMDAR-mediated EPSC with reduced ifemprodil sensitivity. **Conclusions:** These results indicate that while both NR2A and NR2B subunits appear to be present at synapses in the BLA as well as the CeA, the phenotype of the native receptor seems to remain distinct in both nuclei. This may result from differences in the stoichiometry of the subunits in the native receptors in each nucleus, or from post-translational regulation of these subunits in forming receptor complexes.

POS-WED-049

PRINCIPAL NEURONS OF THE BASOLATERAL AMYGDALA RESPOND TO PREFRONTAL CORTEX STIMULATIONS, BUT NOT FOOTSHOCKS, DELIVERED DURING AN UP-STATE

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We have previously shown that the slow oscillation between resting membrane potential (down-state) and depolarized potentials (up-states) that occurs within principal neurons of the basolateral amygdala is strongly influenced by ascending sensory information. For example, footshocks delivered during down-states evoked up-states and reset the phase of the slow oscillation. In contrast to this, footshocks delivered during the up-state had no observable effect. This could be due to an inability of the principal neurons to respond to further afferent stimulation during an up-state. To test this, we used *in vivo* whole cell recording to examine the response of principal neurons to direct stimulation of the medial prefrontal cortex (mPFC), a region that projects directly to the BLA. Stimulation of the mPFC produced a relatively short (181.34 ± 20.21 ms) excitatory post-synaptic response in principal neurons of the BLA. The latency to onset was 27.61 ± 0.8 ms (n=6) and the peak amplitude was 9.83 ± 1.15 mV (n=6). Stimulation of the mPFC during an up-state also elicited an excitatory post-synaptic response, with a latency of 27.41 ± 1.39 (n=4) and the peak amplitude of 4.61 ± 0.75 mV (n=4). This depolarization was always followed by a rapid hyperpolarization (latency of 50.55 ± 4.29 ms; n=4). It is clear from these results that BLA principal neurons can respond to further afferent stimulation during an up-state. As such, we conclude that the lack of a footshock response during an up-state is due to a block of ascending footshock-related information at a level below the BLA.

POS-THU-050

PLATEAU POTENTIALS EVOKED IN BASAL AND APICAL DENDRITES IN LAYER 5 PYRAMIDAL NEURONS OF THE MEDIAL PREFRONTAL CORTEX

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Purpose: The medial prefrontal cortex (mPFC) plays an integral role in higher cognitive functions such as working memory. In vivo studies have shown that mPFC neurons fire repetitively during working memory tasks. To explore what underlies the repetitive firing we examined plateau potentials in layer 5 pyramidal neurons. **Methods:** 300 µm brain slices containing the mPFC were cut from P18–P29 Wistar rats (of either sex) and whole-cell recordings were made from layer 5 pyramidal neurons in the prelimbic mPFC. Plateau potentials were evoked by iontophoresis of glutamate (300 mM, pH 10, 5-1000 ms). **Results:** Neurons were characterized as those that fired action potentials repetitively in response to a depolarizing current injection (n=36) and those that displayed spike frequency adaptation (n=24). No difference in resting membrane potential (-64 ± 4 mV vs -68 ± 1 mV, respectively, p>0.05) or input resistance (62 ± 4 MΩ vs 65 ± 7 MΩ, respectively; p>0.05) was observed between these two groups. Plateau potentials were evoked more readily in repetitive firing neurons (36/36) than in accommodating neurons (10/24). Plateau potentials were also significantly larger and initiated action potentials more readily in repetitive firing neurons. In repetitive firing neurons plateau potentials evoked by glutamate application to apical dendrites were significantly larger than those evoked by glutamate application to the basal dendrites. Plateau potential size evoked in the apical dendrite was independent of the distance from the soma. **Conclusion:** These results show that repetitive firing neurons are more excitable in response to exogenous glutamate than accommodating neurons and therefore may contribute more to the repetitive firing observed during working memory tasks in vivo.

POS-WED-051

PROPERTIES OF VOLTAGE-GATED SODIUM AND POTASSIUM CURRENTS IN NUCLEATED PATCHES FROM LAYER II PRINCIPAL CELLS OF THE MOUSE PIRIFORM CORTEX

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Only two synapses removed from the primary sensory input, superficial pyramidal (SP) cells and semilunar (SL) cells are glutamate-releasing neurons that together comprise the main input layer of the piriform cortex, layer II. These two morphologically-different cell types exhibit distinctive firing and synaptic properties, with likely ramifications for olfactory processing. **Purpose:** Our aim was to compare the biophysical properties of voltage-gated sodium and potassium currents underlying the different firing patterns observed in SP and SL cells. **Methods:** Coronal slices (300 µm thick) containing the piriform cortex were prepared from 17-21 day-old C57/BL6 mice. Firing properties were recorded using whole-cell current clamp. Voltage-clamp recordings of sodium and potassium currents were made from nucleated outside-out patches. **Results:** Application of TEA (200 µM) or 4-AP (200 µM) inhibited burst firing in SP cells with little effect on the firing pattern of SL cells. The TEA-insensitive transient potassium current (I_A) inactivated at more hyperpolarized potentials in SL cells ($V_{1/2}$ = -89.9 ± 0.2 mV, n = 6) than in SP cells ($V_{1/2}$ = -77.1 ± 0.2 mV, n = 4, $p < 0.05$). In contrast, the activation and inactivation properties of voltage-gated sodium currents were not significantly different between the two cell types. **Conclusions:** Differences in voltage-gated potassium channels appear to contribute to the differences in firing properties observed between SL and SP cells. Further work will compare the properties of other classes of potassium channels.

POS-THU-052

DISTINCTIVE CLASSES OF GABA-ERGIC INTERNEURONS PROVIDE PHASE- AND LAYER-SPECIFIC SYNAPTIC INHIBITION IN THE MOUSE PIRIFORM CORTEX

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The piriform cortex (PC) is an anatomically-simple three-layered cortex that processes olfactory information. During olfaction *in vivo*, prominent oscillations in electrical activity are seen in the PC, suggesting that alternating synaptic excitation and inhibition may be critical for the operation of this cortical circuit. **Purpose:** Our aim was to identify the GABAergic interneurons that might provide phasic inhibition in the PC. **Methods:** Experiments used 300 µm-thick coronal slices of PC from GAD67-GFP mice (14-25 days old) in which neurons expressing the GABA synthetic enzyme, GAD67, are labelled with GFP. Targeted whole-cell recordings and immunohistochemistry were accomplished using standard methods. **Results:** We identified five main classes of PC interneurons, based on morphology, laminar location, electrophysiology and expression of molecular markers. The cell classes were: neurogliaform (NG), horizontal (HZ), bitufted (BT), fast-spiking multipolar (fMP) and regular-spiking multipolar (rMP). Each layer contained at least two of these classes, one of which fired earlier in a train of excitatory synaptic inputs, the other later (layer Ia: HZ early and NG late; layer II: NG early and BT late; layer III: fMP early and rMP late; n = 4 for each). Additionally, different classes sent axons to specific regions of layer II principal cells (HZ, NG, rMP: dendrites; BT, fMP: soma). **Conclusions:** These five main classes of GABAergic interneurons in the PC are ideally suited to providing the phasic inhibition that synchronises the electrical oscillations associated with odour processing. Future work will incorporate these data into a model circuit of the PC.

POS-WED-053

POTENTIAL PROPAGATION IN CORTICAL INTERNEURON DENDRITES

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Interneurons are thought to play specific roles in the control of excitation within the cortex. This is achieved through reciprocal synaptic coupling, as well as dendritic gap junctions and dendritic transmitter release. These latter two processes require robust propagation of action potentials (APs) into the dendritic tree. **Purpose:** Here we quantify the efficiency of AP backpropagation (bAP) in cortical interneurons through the novel application of intracellularly injected voltage-sensitive dyes (VSD). This technique allows the direct recording of transmembrane potential simultaneously at multiple dendritic locations, which would be very difficult to achieve with direct electrophysiological methods. **Methods:** Cortical layer 2/3 bitufted interneurons were identified based on their morphology, firing pattern in response to somatic current injection and somatic AP waveform. After identification interneurons were filled with VSD (JPW1114) via the somatic recording pipette, and fluorescent signals generated in response to APs and hyperpolarized steady-state voltage changes measured at multiple dendritic locations. Backpropagating AP fluorescence signals were calibrated at each dendritic location based on the amplitude of the steady-state voltage change estimated from morphologically realistic models. **Results:** On average, bAP signals attenuated to approximately 50% of somatic AP amplitude at dendritic locations 100µm from the soma (n=6). Further investigation will be required to determine if propagation of bAPs into interneuron dendrites is passive, or alternatively is supported by activation of dendritic sodium channels. **Conclusion:** These data show that APs invade the dendrites of cortical layer 2/3 bitufted interneurons in a decremental manner such that their impact on cortical excitability via dendritic gap junctions and dendritic transmitter release would be expected to be greater at proximal dendritic sites.

POS-THU-054

SENSORY DEPRIVATION INCREASES DENDRITIC EXCITABILITY OF LAYER 5 PYRAMIDAL NEURONS IN RAT BARREL CORTEX

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Purpose: Sensory experience is required for the formation and stabilization of cortical maps, with previous work indicating that sensory loss can lead to changes in receptive field properties, synaptic strength, synaptic connectivity, and dendritic morphology of cortical neurons that make up these maps. Surprisingly, there is little evidence for changes in the intrinsic properties of cortical neurons following sensory loss. **Methods**: Here we studied the impact of whisker trimming on the intrinsic membrane properties of layer 5 pyramidal neurons in barrel cortex using somatic and dendritic whole-cell recording in acute brain slices. Whisker trimming was started on postnatal day 3 or 20 (before and after cortical map formation, respectively) and continued until the day animals were sacrificed for slice experiments (postnatal day 21 to 40). **Results**: Active and passive membrane properties assessed at the soma were not different between deprived (n=88) and sham (n=51) conditions ($p>0.05$). Recordings of the dendritic current density of Ih showed a functional down-regulation of HCN channels after sensory deprivation ($p<0.05$) without changes in HCN channels gating properties (sham: n=13; deprived: n=10; $p>0.05$). As a functional consequence, we observed that the critical frequency for dendritic calcium electrogenesis was lower in neurons from animals that had undergone sensory deprivation (~90 Hz, n=21) compared to ~115 Hz in sham controls (n=25, $p<0.05$). This effect is mediated by a functional decrease of Ih current because block of HCN channels lowered the critical frequency to the same value (~70 Hz) for all experimental conditions. **Conclusion**: These results show that dendritic excitability of layer 5 pyramidal neurons is increased following sensory deprivation primarily due to a reduction in dendritic HCN current.

POS-WED-055

ACTIVATION OF NR2B-CONTAINING NMDA RECEPTORS IS REQUIRED FOR LONG-LASTING LTP IN HIPPOCAMPAL AREA CA1

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Long-term potentiation (LTP) is not a unitary phenomenon. In area CA1 of hippocampus, at least 3 different forms of LTP have been shown to co-exist, each involving unique intracellular signalling and effector cascades (Raymond, TINS 30:167-75, 2007). Although most forms of LTP are dependent on activation of postsynaptic NMDA receptors, controversy exists over the relative roles of receptors containing different NR2 subunits. We have investigated the involvement of NMDA receptors containing the NR2B subunit in different forms of LTP at the CA3-CA1 synapse in hippocampal slices from male Wistar rats (7-8 wks). The selective NR2B antagonist Ro 25-6981 (1 µM) had no effect on short-lasting LTP induced by 1 train of theta-burst stimulation (1 TBS, n=3), but dramatically curtailed the persistence of LTP induced by 4 TBS (n=3). Since NR2B-containing receptors constitute a significant extrasynaptic NMDA receptor population, we asked whether extrasynaptic NMDA receptors alone could induce LTP induced by 4 TBS. Synaptic NMDA receptors were inhibited with the use-dependent channel blocker MK-801 (10 µM) during baseline stimulation. Following a 20 min washout of MK-801 in the absence of stimulation during which time synaptic NMDA receptors remain blocked, LTP induced by 4 TBS was inhibited (n=4). Together these data show that long-lasting but not short-lasting LTP requires NR2B receptor activation and that synaptic NMDA receptors are necessary. It remains possible that the requisite NR2B receptors are located extrasynaptically but that they must work in concert with synaptic NMDA receptors. Future experiments will investigate the relative requirements of synaptic and extrasynaptic NMDA receptors.

POS-THU-056

REGULATION OF SYNAPSE FUNCTION BY SYNAPSE-ASSOCIATED PROTEIN 97 (SAP97) ISOFORMS

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The synaptic insertion of GluR1-containing AMPA-type glutamate receptors (AMPARs) is critical for synaptic plasticity. However, mechanisms responsible for GluR1 trafficking and retention at the synapse are unclear. The synapse-associated protein SAP97 has been shown to directly bind GluR1 and to participate in its forward trafficking from the Golgi network to the plasma membrane. Whether SAP97 also plays a role in scaffolding GluR1 at the postsynaptic membrane is unclear, due to its expression as a collection of alternatively spliced isoforms with ill-defined spatial and temporal distributions. In the present study, we have used dynamic imaging and electrophysiology to demonstrate that two postsynaptic, N-terminal isoforms of SAP97 directly modulate the levels, dynamics, and function of synaptic GluR1-containing AMPARs. Specifically, the unique N-terminal domains confer distinct subsynaptic localizations onto SAP97, targeting the palmitoylated alpha isoform to the postsynaptic density (PSD) and the L27domain-containing beta isoform primarily to non-PSD, perisynaptic regions. Furthermore,while both isoforms promote the postsynaptic, cell-surface accumulation of GluR1, they differentially influence GluR1 dynamics and function, with alphaSAP97 enhancing and betaSAP97 inhibiting AMPAR-mediated currents. These results indicate that N-terminal splicing of SAP97 can control synaptic strength by regulating AMPAR responsiveness to glutamate.

POS-WED-057

α_1-ADRENERGIC MODULATION OF TRANSMITTER RELEASE IN LAYER II/III PYRAMIDAL NEURONES IN SOMATOSENSORY CORTEX OF RAT

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Presynaptic Ca^{2+} stores modulate transmitter release in layer II/III neurones in rat somatosensory cortex. This modulation can result from IP_3 production caused by presynaptic metabotropic glutamate receptors[3] (mGluR5). **Purpose**: To test whether presynaptic α_1-adrenergic receptors have the potential to cause Ca^{2+} mobilization from stores, as they are also linked to PIP_2 hydrolysis and the resultant IP_3 production. **Methods**: 300 µm thick parasagittal slices were prepared from 15-19 day old Wistar rats. Miniature excitatory postsynaptic currents (mEPSCs) were recorded in layer II/III cells, which were later histologically verified as pyramidal neurones. Voltage-clamp recordings were obtained at 36±1°C in the presence of tetrodotoxin (1 µM) and gabazine (3 µM). α_1-adrenergic receptors were activated by noradrenaline (NA; 10 µM), cirazoline (CO; 5 µM) or blocked by prazosin (PA; 30 µM). Superfusion rate was 4 ml/min. **Results**: Pyramidal neurones show an average mEPSC amplitude of -10.7±0.6 pA and rate of 28±4 Hz (n=12). Application of NA (n=12) increased the rate by 34±8%, but had little effect on amplitude. Addition of CO (n=18) increased the frequency by 13±3%; PA (n=6), however, had no significant effect on either parameter. The increase in mEPSC frequency decayed within an additional 10 min of agonist application by 9±0.9% (n=5). **Conclusion**: We have shown that presynaptic α_1-adrenoreceptors are functionally capable of increasing mEPSC frequency without affecting amplitude. Unlike mGluR5, these receptors seem not to be tonically activated. Future experiments are still required to directly link adrenergic IP_3 production with Ca^{2+} release from stores. We postulate 1) that the decrease in mEPSC after initial agonist application may be the result of either receptor desensitization or internalization and 2) that presynaptic α_1-adrenergic receptor activation can change evoked transmitter release dynamics. [3]C. R. L. Simkus & C. Stricker, J. Physiol. **545**, 521 (2002).

POS-THU-058

THE HYPERPOLARISATION ACTIVATED CURRENT (I_H) SIGNIFICANTLY CONTRIBUTES TO THE AFTERHYPERPOLARISATION IN STRIATAL CHOLINERGIC INTERNEURONS

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Purpose: The tonically active cholinergic neurons in the striatum have been shown to develop a pause response to sensory stimuli that are associated with a reward. We have hypothesised that these firing pauses are caused by an afterhyperpolarisation (AHP) and aimed to characterise the AHP following cortical synaptic inputs. **Methods:** Whole-cell patch-clamp recordings were made from striatal cholinergic interneurons using rat brain slices. Successively larger depolarising postsynaptic potentials (PSPs) were evoked using repetitive stimulation of corticostriatal fibres at 60-120 Hz. **Results:** The magnitude of the AHP was directly proportional to the magnitude of the preceding membrane depolarisation in all neurons tested (n=24). Calcium-activated potassium currents expressed in cholinergic neurons may contribute to this AHP. However, apamin antagonism of SK-type channels (n=6) and calcium depletion from intracellular stores (n=5) had no effect on the synaptically induced AHP. As the AHPs were triggered efficiently over a wide range of membrane potentials negative to -50 mV we examined if the AHP following subthreshold synaptic stimulation is mediated by the hyperpolarisation activated mixed cation current (I_h). Blocking I_h with 2 mM Cs^+ (n=7) or 30-50 µM ZD7288 (n=8) greatly reduced the size of the AHP and the proportion of episodes that contained a measurable AHP at larger PSP magnitudes. The measured reversal potential near -20 mV and voltage dependence of the synaptically induced AHP current agree with the characteristics of I_h. **Conclusion:** Afferent synaptic potentiation as well as regulatory mechanisms that modulate Ih channel activity may underlie the appearance of pauses in tonic firing of cholinergic interneurons which develop during learning.

POS-WED-059

GLYCINERGIC INHIBITORY INPUT ONTO BUSHY CELLS IN THE COCHLEAR NUCLEUS

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Purpose: Neurons within the cochlear nucleus, spherical bushy cells (SBCs) and globular bushy cells (GBCs), are important for sound localisation as they initiate the pathways that encode interaural time and level differences, respectively. As part of a plan to investigate the role of inhibition in sound localisation pathways, we have quantified the inhibitory glycinergic input onto bushy cells. **Methods**: Double labelling with cresyl violet and glycine immunohistochemistry was used to identify bushy cell types and their glycinergic inputs, respectively. Fluorescent confocal microscopy was used to investigate the three-dimensional distribution of glycinergic inputs onto SBC and GBC cell somata. Quantification of immunopositive glycine synaptic terminals was performed in sagittal sections (50 µm) of cochlear nucleus using camera lucida at the light microscope level. **Results:** Three-dimensional reconstructions indicated that glycinergic inputs were distributed similarly on the cell somata of SBCs and GBCs. GBCs, however, showed a significantly greater number of glycine terminals (19 ± 0.8; n=15) than spherical bushy cells (13 ± 0.9; n=15; $p<0.01$; unpaired t test). This difference was maintained when the number of terminals was standardised to cell circumference for GBCs (0.4 ± 0.02 terminals/µm) and SBCs (0.3 ± 0.02 terminals/µm; $p<0.01$). **Conclusion:** The results suggest differing roles for glycine in the initiation of auditory pathways encoding interaural time and level differences. Future studies will combine these anatomical findings with electrophysiological recordings of inhibitory responses in these cells.

POS-THU-060

DISCOVERING NEW DRUGS TARGETING GLYCINE RECEPTOR CHLORIDE CHANNELS

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Purpose: The Glycine receptor (GlyR) chloride channel mediates inhibitory neurotransmission in the spinal cord, brain stem and retina. Most glycinergic neurotransmission is mediated by α1β GlyRs although α3β GlyRs are also present in inhibitory synapses on spinal pain sensory neurons. Inflammatory pain sensitisation is caused by PGE2¬-mediated down-regulation of α3 mediated glycinergic currents in nociceptive neurons. Thus, compounds that selectively potentiate α3-containing GlyRs could produce analgesia. This study reports our progress in identifying candidate lead compounds. **Methods:** Extracts from marine organisms were screened against α1 and α3 GlyRs stably expressed in HEK293 cells using an anion-sensitive yellow fluorescent protein assay. The potencies of 22 novel pure compounds present in active fractions were quantitated at α1 and α3 GlyRs by automated patch-clamp electrophysiology. **Results:** Compound 10 dramatically potentiated α3 GlyRs (EC50= 8.5 ± 2.1 µM; n = 6) but had no effect on α1 GlyR. Three other novel compounds strongly potentiated α1 GlyRs, with two of them exhibiting submicromolar potencies (all n = 6 cells). **Conclusion:** Compound 10 exhibited the appropriate pharmacological profile for an α3 GlyR-targeted anti-inflammatory analgesic, and is our most promising lead so far. We are currently screening analogies of successful test compounds in an attempt to improve potency.

POS-WED-061

A NOVEL PEPTIDE ISOLATED FROM CONE SNAIL VENOM IS ACTIVE AT THE NEUROTENSIN RECEPTOR

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Purpose: Neurotensin (NT), a 13 amino acid peptide, is both a neurotransmitter and a gastrointestinal regulatory peptide. In the CNS, NT plays a role in hypothermia, antinociception, control of anterior pituitary hormone secretion, muscle relaxation, and interacts with the dopamine system. One NT receptor agonist, contulakin-G, has been identified, to date, in cone snail venom (Craig *et al.*, 1999). The aim of the present study was to isolate and identify peptides from the venoms of Australian cone snails that are active at the NT receptors. **Methods:** Cone snails were collected from the Great Barrier Reef and the venom was extracted from the venom ducts using aqueous acetonitrile acidified with acetic acid. Crude venom was tested for activity in a radioligand binding assay using rat brain preparations. **Results:** An active peptide was isolated from the venoms by RP-HPLC and characterized by mass spectrometry. The peptide is different to contulakin-G and has not been described previously. The venom fraction containing the peptide displaced NT in radioligand binding assays using rat brain preparations and COS-7 cells expressing rat or human neurotensin receptor subtype 1 (NTR1). The binding affinity of the *Conus* peptide for rat and human NTR1 was the same order of magnitude as that of NT (i.e. nanomolar). Radiolabelled NT did not bind to non-transfected COS-7 cells. The *Conus* venom fraction containing the peptide was also able to stimulate cAMP release in a dose-dependent manner in COS-7 cells expressing hNTR1. **Conclusion:** Further characterization of the peptide will be possible, once it has been sequenced and synthesized. New agonists and antagonists for specific NT receptors will be useful pharmacological tools for assessing the functions of each receptor subtype. Craig *et al.* (1999) J. Biol. Chem. 274: 13752-13759.

POS-THU-062

TRYPTOPHAN DEFINES A KEY INTERACTION BETWEEN MU O-CONOTOXIN MRVIB AND THE VOLTAGE-GATED SODIUM CHANNEL

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Purpose:μO-conotoxin MrVIB is a 31 residue peptide isolated from the venom of the predatory cone snail *Conus marmoreous*. In this study, the synthesis and functional characterization of synthetic MrVIB and a point mutation [W6A]MrVIB were undertaken to evaluate the contribution of this tryptophan to channel selectivity and affinity. Methods: Recombinant $Na_v1.2$ and $Na_v1.8$ channel subunits were expressed in Xenopus oocytes and Na^+ currents evoked by a step depolarization from -70 mV to +0 or +10 mV, respectively, using an automated workstation with eight channels in parallel, including drug delivery and on-line analysis (OpusXpress ™ 6000A). Results: Native MrVIB (100 nM) inhibited $Na_v1.8$-mediated currents by 38 ± 6 % (n = 6) and $Na_v1.2$ currents by 12 ± 5 % (n = 4) of control. The active MrVIB 2-5 isomer II inhibited the $Na_v1.8$ current by 35 ± 5 % (n = 8) and $Na_v1.2$ current by 9 ± 2 % (n = 3). Concentration-response curves for MrVIB 2-5 isomer inhibition of Nav1.8 channels revealed an IC_{50} value of 140 ± 11 nM (n = 4) which was comparable to the native MrVIB activity (110 ± 18 nM, n = 5). Replacement of tryptophan with alanine completely abolished activity at both $Na_v1.2$ (n = 4) and $Na_v1.8$ (n = 4) channel subtypes. Conclusions: An improved synthetic methodology for MrVIB was achieved with the synthetic isomer having similar physiological properties to the native MrVIB, which has enabled us to identify tryptophan as an essential residue for MrVIB activity at Na_v channels.

POS-WED-063

ACTIVATION MECHANISM OF THE α1β2 GABA-A RECEPTOR PROBED USING VOLTAGE-CLAMP FLUOROMETRY

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Purpose: GABA-A chloride channel receptors mediate most inhibitory neurotransmission in the central nervous system. The loop F of α1 subunit ligand-binding domain forms part of the GABA binding site, and previous studies predicted this domain might be involved in channel activation. This study tested this by employing voltage-clamp fluorometry to monitor the conformational change induced by different agonists and antagonists in loop F of the α1 subunit. **Methods**: We generated the R186C mutation in loop F of the α1 subunit. GABA-A receptors comprising α1□R186C and β2 subunits were then recombinantly expressed in Xenopus oocytes. These were studied using simultaneous voltage-clamp and micro-fluorometry. Oocytes were surgically removed from anaesthetized frogs by procedures approved by the University of QLD Animal Ethics Committee. Results: We successfully labelled R186C with the fluorophore MTS-Rhodamine, and monitored the fluorescence change induced by different agonists (GABA, TACA and β-alanine), competitive antagonist (SR-95531) and modulator (Diazepam). All agonists decreased fluorescence by 4%-5% (n ≥ 4 oocytes for each). SR-95531 evoked the same direction of fluorescence change by 6% (n = 4). Although diazepam modulated current, it did not produce any fluorescence change at α1-R186C. **Conclusion**: Because agonists and antagonists produced similar changes at this position, we conclude that the α1 subunit F loop is not involved in receptor activation. It is more likely to be involved in locking ligands onto the binding site.

POS-THU-064

CHARACTERISATION OF EXCITATORY AMINO ACID TRANSPORTER 2 SPLICE VARIANTS IN A FLUORESCENCE-BASED MEMBRANE POTENTIAL ASSAY

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Purpose: Excitatory Amino Acid Transporter 2 (EAAT2) is the major glutamate transporter in the CNS. Failure to reduce extracellular glutamate levels after synaptic transmission can lead to excitotoxicity via overstimulation of post-synaptic glutamate receptors. Several exon-splice variants of EAAT2 have been found in healthy human and Alzheimer's diseased brains. Relative expression of two exon-skipping variants (EAAT2 Δ7 and EAAT2 Δ9) was increased in AD; with increasing pathological severity. This could lead to a reduced ability to transport glutamate, either by inhibiting the trafficking of the transporter to the plasma membrane or by disrupting the assembly of the transporter complex. **Methods:** Glutamate uptake of EAAT2 splice variants was investigated using a fluorescence-based membrane potential assay (FMP). HEK293 cells were transiently transfected with different ratios of EAAT2 wt to EAAT2 splice variants and assayed on an automated fluorescence microscope. **Results:** EAAT2wt transfected cells exposed to L-glutamate showed a concentration-dependent increases in fluorescence intensity (EC_{50}: 32 μM ± 1.2). This data is in correlation with electrophysiology studies (34 μM ± 6). Dependent on the amount of transfected EAAT2 wt DNA (0.1μg to 5μg) EC_{50} values did not change. Similar results where obtained for EAAT2b at different EAAT2b:EAAT2wt ratios. No significant fluorescence response was measured in untransfected and EAAT2 exon skipping variant transfected cells. EAAT2wt and splice variants co-transfections at different ratios (1:5 for EAAT2 Δ7 and 1:20 for EAAT2 Δ9) increased EC_{50} values 20-40 times in comparison to EAAT2wt (EAAT2 Δ7: 775μM ± 5; EAAT2 Δ9: 1300μM ± 5). **Discussion:** EAAT2 exon-skipping splice variants have lower glutamate transport capabilities and could therefore cause excitotoxicity.

POS-WED-065

DYNAMIC CLAMP ANALYSIS OF ACTION POTENTIAL FIRING PROPERTIES IN A MOUSE MODEL OF FAMILIAL EPILEPSY

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Purpose: Standard electrophysiological probing of input/output relationships generated by prolonged and large current steps do not adequately recapitulate physiological synaptic inputs and the conductance changes seen in vivo. As such, protocols based on these techniques may not have the necessary sensitivity to detect changes in neuronal excitability in disease states. To address this we have implemented dynamic clamp controlled synaptic input to probe properties from normal and epileptic neurons. **Methods**: We modeled the *in vivo* properties of excitatory and inhibitory conductances together with a noise term using the Ornstein-Uhlenbeck process and implemented real time feedback using The MathWorks Simulink and xPC Target modules. Step protocols that incrementally varied conductance and noise were then introduced into hippocampal pyramidal neurons from control and an epilepsy mouse model. **Results**: We compared input-output (I-F) curves generated by current step protocols with dynamic clamp protocols (n=5). The dynamic clamp analysis revealed features that were not seen with current steps, including a highly variable AP amplitude that fired irregularly. This differed from the stereotypical patterns noted with traditional current step protocols. These relationships provide additional information about neuronal excitability. Further analysis will assess latency and probability of firing in different conductance states and with different noise levels to compare neuronal properties in normal and disease states. **Conclusions**: Synaptic noise and conductance delivered by dynamic clamp may provide a more realistic input on which to determine neuronal excitability. We intend to use this approach to dissect out subtle neuronal excitability changes seen in genetic models of epilepsy and relate these to disease genesis.

POS-THU-066

TRANSIENT TEMPERATURE ELEVATION INCREASES GABA-MEDIATED INHIBITION IN LAYER 2/3 CORTICAL NEURONS IN A MOUSE MODEL OF FAMILIAL EPILEPSY

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Purpose: A GABAA receptor γ2 subunit mutation (R43Q) is associated with Febrile Seizures (FS) in a large Australian family. Recent data from heterologous expression systems suggest that temperature-dependent internalization of GABAAγ2(R43Q) receptors is the mechanism underlying FS genesis in these families. We have previously reported on the creation of a knockin mouse model harboring the same mutation and asked whether a similar temperature dependent change in mutant receptor internalization also occurs in our model. **Methods:** Miniature inhibitory postsynaptic currents (mIPSCs) were recorded from layer 2/3 cortical pyramidal neurons in brain slices taken from heterozygous animals (RQ) and wild type (RR) littermates: slices were pre-incubated for 1hr at 38°C or 22°C prior to recording at 34°C. Total γ2 subunit containing receptors were determined in whole brains from heated or non-heated animals using [3H]flumazenil radioligand binding. **Results:** Preheating slices from RQ mice increased both the mIPSC amplitude and fast deactivation (15 preheated and 11 RT cells; $p<0.05$) whereas no significant effect was observed in wild type (20 preheated and 10 RT cells; $p>0.3$). [3H]flumazenil binding in whole brain homogenates was reduced in heterozygous samples (n=8) compared to wild type (n=9; $p<0.001$) but displayed no temperature dependent changes for either genotype ($p>0.05$). **Conclusion:** Although data from heterologous expression systems predicted a decrease in mIPSC amplitude by temperature-dependent receptor internalization of mutant receptors, we saw no evidence of receptor internalization from cortical pyramidal neurons of the RQ mouse. Therefore, our data not only highlight the differences in behaviour of cell biological processes in heterologous and native systems but, importantly, suggest that other mechanisms are responsible for FS genesis in these families.

POS-WED-067

EVIDENCE FOR PRESYNAPTIC NMDA RECEPTORS IN MEDIATING EXCITATORY NEUROTRANSMISSION IN THE SPINAL TRIGEMINAL NUCLEUS

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Purpose: We have previously reported that increased vesicular glutamate release following purinergic (P2X) receptor activation in the spinal trigeminal nucleus caudalis (also referred to as the medullary dorsal horn, MDH) is dependent on NMDA receptor activation. The aim of the current study is to investigate the location of these NMDA receptors and how they potentiate transmitter release. **Methods:** Sprague-Dawley rat pups (P7-21 days) were anaesthetised with halothane, decapitated and horizontal slices (250μm) were cut from the caudal brainstem. Whole-cell patch-clamp recordings (voltage clamped at –70mV) were made from MDH neurons and mEPSCs and evoked paired-pulse recordings were analysed. **Results:** The current study suggests that the NMDA receptors involved are not located on the postsynaptic neuron since the inclusion of MK-801 (1mM, NMDA open-channel blocker) in the pipette solution did not alter the α,β-meATP-induced increase in glutamate release (n=11, $p<0.05$). Furthermore, superfusion of NMDA (100μM) and D-serine (10μM, n=10) caused an increase in the ratio of response amplitudes evoked by paired-pulse stimulation, suggesting activation of presynaptic NMDA receptors. In addition, the α,β-meATP-induced increase in paired-pulse ratio (PPR) was not altered following 30 minute pre-incubation with Fluoroacetate (1μM, glial aconitase inhibitor, n=6, $p<0.005$). Activation of NMDA receptors may involve a phosphorylation event as the PPR increase following α,β-meATP (30μM) was abolished in the presence of either the PKC inhibitor, Chelerythrine (5μM, n=6, $p>0.5$) or PKA inhibitor, H-89 (2.5μM, n=6, $p>0.5$). **Conclusion:** These results suggest that NMDA receptors mediating the increase in neurotransmission are located on presynaptic afferent terminals and not on astrocytes. These data indicate that presynaptic NMDA receptors play a fundamental role in regulating transmitter release at this synapse.

POS-THU-068

EFFECTS OF NEUROKININ AND CANNABINOID RECEPTOR ACTIVATION ON THE EXCITABILITY OF NEURONS IN PELVIC GANGLIA OF FEMALE MICE

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Neurons in pelvic ganglia relay information from the CNS to pelvic organs including reproductive tract, bladder, and lower bowel. The endogenous cannabinoid anandamide, and the neurokinin substance P have been shown to alter excitability of some autonomic neurons. Consequently they may provide a mechanism for modulation of pelvic organ function. **Purpose.** In this study we investigate the effects of NK and CB1 receptor activation on the excitability of neurons in pelvic ganglia of female mice. **Methods.** Mice (C57/B16) were killed under deep anaesthesia (inhaled isoflourane, 5% in air) prior to removal of the pelvic plexus. Membrane potential and current were recorded using intracellular microelectrodes. The location of NK1 receptors was investigated using immunohistochemistry. **Results.** The afterhyperpolarization (AHP) following an action potential, and the underlying current (IAHP) was markedly reduced by 100 μM Cd^{2+} in 4 of 5 neurons indicating a role for calcium activated potassium channels. 1 μM SP depolarised or evoked an inward current in 3 of 7 neurons, but had no effect on the amplitude or duration of the AHP. NK1 receptors were expressed by < 5% of neurons in pelvic ganglia (n=3 mice). 5 μM methanandamide had no effect on membrane potential or the AHP (12 neurons tested). In 4 of 4 neurons tested, action potential half width was decreased in the presence of methanandamide. **Conclusions.** These results indicate that activation of NK1 and CB1 receptors affect only a small proportion of neurons in pelvic ganglia. Whether these neurons represent functionally related populations remains to be tested.

POS-WED-069

THE EFFECT OF LAVENDER AND TRANS-2-HEXENAL ON SMOOTH MUSCLE

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Lavender and *trans*-2-hexenal are plant-derived chemicals commonly found in many household products, as well as being widely used in complementary medicine. However, their effects on the nervous system and muscles has not been well characterised. **Purpose:** The present study examined the dose-dependent effects of lavender and *trans*-2-hexenal on neurotransmitter release from sympathetic varicosities and their effect on smooth muscle. **Methods:** Extracellular recording of excitatory junction currents (EJC) and Nerve Terminal Impulses (NTI) generated from the mouse ductus deferens was used to determine the effects of these chemicals on neuromuscular transmission. **Results:** Lavender and *trans*-2-hexenal decreased the EJC in a dose-dependent manner (range: 0.001% - 0.05%) and at a concentration of 0.05% abolished the amplitude of the EJC (n=6) while still recording the NTI (n=6). This reduction in EJC amplitude was not reversed by paired pulse facilitation or by increasing the extracellular calcium concentration, suggesting that these chemicals do not mediate their effects by reversibly inhibiting voltage-gated calcium channels (VGCC). This is an important finding as previous research in this field had not clarified whether Lavender decreased neurotransmission by reversibly or irreversibly blocking VGCCs. At lower concentrations *trans*-2-hexenal was shown to affect fast and slow EJCs differently, suggesting that *trans*-2-hexenal may uncouple smooth muscle cells. **Conclusion:** Our present findings indicate that both lavender and *trans*-2-hexenal relax smooth muscle.

POS-THU-070

EXPRESSION AND FUNCTION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN SENSORY NEURONS INNERVATING THE TEMPOROMANDIBULAR JOINT (TMJ)

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Purpose: The aim of this study was to establish a behavioural model of TMJ sensitivity to inflammation, and to determine whether peripheral NMDA receptors contribute to the sensitivity. **Methods:** In one series of experiments, adult male rats were anaesthetised with isoflurane, and the TMJ injected with 4µl of complete Freund's adjuvant (CFA; 1µg bacterium) and vehicle, vehicle alone, or CFA and the NMDA antagonist AP5 (40µM). Sensitivity was tested with calibrated von Frey filaments, applied over the TMJ, 1 day pre- and 1 and 2 days post-injection. We also tested sensitivity to CFA alone (n=10) and vehicle alone (n=9) for up to 3 weeks after injection to establish that peak sensitivity was at 1 day. In another series of experiments, the TMJ was injected with 2% Fast Blue, under ketamine anaesthesia, and perfused 7 days later with 4% paraformaldehyde. The trigeminal ganglia were removed, 12 µm sections cut on a cryostat and one section every 100µm section processed for NMDA receptor subunit NR1 and NR2B immunoreactivity. **Results:** There was a significant increase in sensitivity to CFA at day 1 post-relative to pre-injection (n=7; $P<0.05$; Mann-Whitney test). AP5 blocked this increase in sensitivity (n=7). Of the neurons retrogradely labelled with Fast Blue (178 neurons), 97±0.8% (mean±SEM; n=4) expressed NR1 and 75±0.9% (n=4) expressed NR2B. **Conclusion:** These results indicate that peripheral application of the NMDA antagonist AP5 can prevent behavioural sensitisation following inflammation of the TMJ and that most trigeminal neurons innervating the TMJ express NMDA receptors. This highlights the importance of NMDA receptors in primary afferent excitability.

POS-WED-071

MECHANISMS MODULATING OF SLOW-WAVE ACTIVITY IN ISOLATED MOUSE SMALL INTESTINE

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Purpose: Motility in the mouse small intestine is strongly influenced by myogenic slow wave activity generated by the interstitial cells of Cajal (ICC), however less is known about the mechanisms modulating slow waves. The aim of this study was to determine whether slow waves are modified by neural activity or by the presence of nutrients in the lumen. **Methods:** Isolated segments of mouse (C57B/6) small intestine (duodenum, and jejunum) were placed in an organ bath, cannulated and luminally perfused with physiological saline containing decanoic acid (300 µM) and L-phenylalanine (50 mM). In a separate set of experiments tetrodotoxin (TTX, 1 µM) alone was added to the bath solution with saline in the lumen (duodenum, jejunum and ileum). Slow-wave activity was monitored by constructing spatiotemporal maps of circular muscle diameter from video-recordings of each segment. Slow waves appeared as small high frequency (28.8 - 44.4 min^{-1}) constrictions. **Results:** Frequency of slow wave contractions showed a regional gradient decreasing from duodenum (42.3 ± 2.0) > jejunum (36.1 ± 3.8) > ileum (35.1 ± 5.1, n>10, $p<0.01$), and propagated anally regardless of any association with a neurogenic propagating contraction complex. Slow wave frequency was significantly reduced in duodenum (35.4 ± 0.2, n=3) and jejunum (30.3 ± 0.0, n = 4) by TTX ($p<0.0001$ in each case). In contrast, the frequency of slow wave contractions was increased in duodenum (47.7 ± 3.6) and jejunum (40.9 ± 5.9) when decanoic acid and L-phenylalanine were both present in the lumen (in each region $p<0.0001$, n=4). **Conclusion:** Slow wave activity mouse small intestine is modified by neural mechanisms and upregulated in the presence of nutrient.

POS-THU-072

CHOLERA TOXIN INDUCES SUSTAINED HYPEREXCITABILITY IN MYENTERIC AH NEURONS IN GUINEA-PIG JEJUNUM

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Purpose. Cholera toxin (CT) causes hypersecretion across the intestinal mucosa by activating neural pathways within the enteric nervous system. In guinea-pig jejunum, pre-incubation with CT causes sustained hyperexcitability in specific classes of secretomotor neurons contained within the submucosal plexus. The aim of this study was to determine whether pre-incubation with CT has long-term effects on the excitability of afferent neurons in secretomotor pathways activated by CT. **Methods.** Isolated segments of guinea-pig jejunum were infused with CT (12.5 µg/ml, 0.25-0.4 ml) or saline, tied at each end and incubated for 1.5 hours at 35°C. The tissue was then dissected to reveal the myenteric plexus circumferentially adjacent to intact mucosa and pinned into an organ bath. Standard intracellular recording techniques were used to examine synaptic inputs and excitability of AH neurons (thought to be intrinsic sensory neurons) in ganglia next to the mucosa. Impaled neurons were injected with biocytin and later processed to reveal their morphology. **Results.** 19 AH neurons (7 control, 12 CT-treated) were analysed. During the injection of depolarizing current pulses (0.5 – 3.5 nA), AH neurons from CT-treated preparations fired significantly more action potentials (1.0 nA current pulse: con 0.6 ± 0.3, CT 2.1 ± 0.6; p=0.04) and for significantly longer (1.0 nA: con 27.6 ± 13.4 ms, CT 81.3 ± 18.6 ms; p=0.04) than control AH neurons. CT did not affect the resting membrane potential, input resistance or synaptic potentials of hyperexcitable AH neurons. **Conclusions.** Pre-incubation with CT induces sustained hyperexcitability in myenteric AH neurons next to intact mucosa in guinea-pig jejunum. CT induced hypersecretion might occur as a result of hyperexcitability in both afferent and efferent neurons of secretomotor pathways.

POS-WED-073

THE EFFECT OF CHRONIC MORPHINE TREATMENT ON VESICULAR ASSOCIATED PROTEINS

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Chronic morphine treatment (CMT) has been shown to increase the release of neurotransmitter. Different mechanisms have been proposed to account for this increase including changes in the co-localisation of vesicular associated proteins (VAPs) and changes in phosphorylation of VAPs. **Purpose:** In this study, the effect of CMT on co-localisation of key VAPs and their phosphorylation states has been compared and correlated with transmitter release levels. **Method:** Mice, 4 week old were treated with morphine (100 mg/kg) three times per day for 10 days (CMT). Control animals (CST) received saline injections (subcutaneously). Following treatment animals were anaesthetised with CO_2 and sacrificed by cervical fracture. One vas deferens was used for electrophysiological examination of transmitter release during 0.2 and 4 Hz nerve stimulation and the other vas deferens was used for immunohistochemical examination using various VAP-antibodies. The VAPs examined were SV2, synaptophysin, synaptotagmin 1 and phosphorylated and dephosphorylated synapsin I. **Results:** Transmitter release from CMT vasa deferentia was 46 +8% higher when compared to CST vasa deferentia. The level of co-localisation between SV2 and other VAPs was increased in CMT vasa deferentia (n=8). There is an increase in the ratio of phosphorylated/dephosphorylated synapsin 1 in the CMT vasa deferentia. This correlated well with the increase in transmitter release during 0.2 Hz stimulation and the greater depression in transmitter release during 4 Hz stimulation. **Conclusion:** The increase in transmitter release induced by CMT and acute withdrawal may be mediated by enhanced co-localisation of VAPs and changes in vesicle availability brought about by the phosphorylation state of synapsin I.

POS-THU-074

FUNCTIONAL CONSEQUENCES OF CAMKII PHOSPHORYLATION AT THR253 IN NEURONS

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Purpose: Calcium/calmodulin stimulated protein kinase II (CaMKII) is an important regulator of synaptic function. The biological properties of CaMKII are regulated by multi-site phosphorylation and targeting to cellular microdomains through interactions with proteins. The roles of two phosphorylation sites, Thr286 and Thr305/6, have been well characterised. We have identified a new phosphorylation site at Thr253. Evidence from rat brain *in vivo* that phosphorylation of Thr253 is dynamically regulated independently of Thr286 phosphorylation following synaptic stimulation and in three models of neurological disease, implies that Thr253 phosphorylation regulates specific functions. This study aims to identify some of the functional effects of Thr253 phosphorylation using neuronal cell lines transfected with phospho-mimic mutants of CaMKII. **Methods:** Neuronal cells were transfected with either a doxycycline-inducible vector system or a CMV-driven promoter vector system expressing recombinant αCaMKII (wild-type and phospho-mimic mutants Thr253Asp or Thr286Asp). Cellular morphology and growth rates were measured following CaMKII expression. **Results:** Following the transfection of SHSY5Y neuroblastoma cells with recombinant CaMKII (n>2), Thr286Asp (n>2), or Thr253Asp (n>5), morphological and growth rate changes were observed that varied depending on the type of phospho-mimic CaMKII expressed. Transfection with either Thr286Asp or Thr253Asp reduced cell growth but the effect was considerably greater with Thr253Asp and the morphology of the cells was dramatically altered following Thr253Asp transfection. **Conclusions:** These results strongly suggest that phosphorylation of CaMKII at Thr253 is involved in regulating cell growth and morphology independently of phosphorylation at Thr286.

POS-WED-075

SPONTANEOUS SYNAPTIC ACTIVITY IN RETINAL GANGLION CELLS IS MODULATED BY ENDOCANNABINOIDS

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Endocannabinoids and their receptors have been localised to all retinal cells. The endocannabinoid system plays an important role in short term plasticity of excitatory and inhibitory synaptic activity in the CNS. Upon depolarisation of postsynaptic neurones, cannabinoids are synthesized on demand and travel in a retrograde fashion to activate presynaptic cannabinoid receptors (CB1R), which in turn reduce neurotransmitter (NT) release. These mechanisms are postulated to modulate neuronal excitability. The effect of cannabinoids in the retina has been documented in photoreceptors, horizontal bipolar and ganglion cells (RGCs) and in spontaneous synaptic activity in cultured amacrine cells. The effect of cannabinoids on spontaneous synaptic activity in RGCs has not yet been characterised. **Purpose**: To investigate the effects of cannabinoids on spontaneous synaptic activity in RGCs. **Methods**: Whole cell patch clamp recordings from young and adult mouse RGCs were carried out in the whole mount preparation. Inhibitory and excitatory synaptic currents were isolated by using specific antagonists. Spontaneous synaptic currents were recorded before and after the administration of a CB1 cannabinoid receptor agonist WIN55212-2 (5μM). **Results**: WIN55212-2 reduced inhibitory as well as excitatory spontaneous synaptic activity in RGCs; this effect was due to a reduction in the frequency, where as the amplitude of currents remained unchanged (N=32). Excitatory and inhibitory synaptic currents were significantly reduced in RGCs from adult mice when WIN55212-2 was added as well as excitatory currents in young RGCs from mice ($p<0.05$). The effect of CB1 agonist on inhibitory currents in RGCs from young mice while showing a similar trend was not significant. **Conclusion**: Cannabinoid agonists reduce NT release from *both* GABA and glutamate, demonstrating that the endocannabinoid system gates RGC activity suggesting that it might be involved in some forms of short term plasticity modulating signal transmission in the retina.

POS-THU-076

ENDOCANNABINOID SIGNALLING IN THE PERIAQUEDUCTAL GRAY VIA THE MUSCARINIC CHOLINERGIC SYSTEM

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Purpose: Endogenous cannabinoids (endocannabinoids) play a critical role in modulating a number of functions within the midbrain periaqueductal gray (PAG), particularly the control of pain and analgesia. Importantly, it has recently been shown that endocannabinoids functionally mediate the non-opioid component of stress-induced analgesia (SIA). However, the precise cellular mechanisms underlying endocannabinoid signaling in the PAG are unknown. Recent studies in other brain regions have shown that endocannabinoids can be released retrogradely from neurons via depolarisation and/or activation of certain G-protein-coupled receptors, where they generally suppress GABAergic transmission. The present study aimed to examine whether these same mechanisms are involved in the induction and synthesis of endoocannabinoids within the PAG. **Methods:** Whole cell patch-clamp electrophysiological recordings of PAG neurons were conducted from rat midbrain slices. **Results:** The cholinergic agonist, carbachol reduced the amplitude of evoked inhibitory and excitatory postsynaptic currents (eIPSCs and eEPSCs). Carbachol increased the paired-pulse ratio of evoked IPSCs and EPSCs, and reduced the rate, but not the amplitude of spontaneous miniature IPSCs. The carbachol inhibition of evoked IPSCs was mimicked by the acetylcholinesterase inhibitor, physostigmine and was reduced by the M1 and M1/M3 muscarinic cholinergic receptor (mAChR) antagonists, pirenzepine and 4-DAMP, but not by the M2 and M4 antagonists, gallamine and PD-102807. The carbachol inhibition of evoked IPSCs was reduced by the cannabinoid CB1 receptor antagonist, AM251 and the DAG lipase inhibitor, tetrahydrolipstatin and was abolished in the combined presence of AM251 and gallamine. The carbachol inhibition of evoked EPSCs was also reduced in the presence of both gallamine and AM251. **Conclusions:** The current results demonstrate that similar to other brain regions, endocannabinoid signaling can occur in the rat PAG through activation of G-protein coupled receptors, specifically Gq-coupled M1/M3 muscarinic receptors.

POS-WED-077

SUBSTANCE P DRIVES LOCAL NETWORK-MEDIATED ENDOCANNABINOID SIGNALING IN THE PERIAQUEDUCTAL GREY

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Purpose: Substance P is thought to play an essential role in several forms of supraspinally-mediated analgesia. The actions of substance P on synaptic transmission within descending analgesic pathways, however, are unknown. **Methods:** We used whole-cell recordings from rat midbrain slices to examine the effects of substance P on GABAergic and glutamatergic transmission within the periaqueductal grey (PAG), a key component of a descending analgesic pathway that projects via the rostral ventromedial medulla (RVM) to the spinal cord dorsal horn. **Results:** Substance P reversibly decreased the amplitude and increased the paired-pulse ratio of evoked IPSCs recorded from identified PAG-RVM projection neurons and from unidentified PAG neurons. In contrast, substance P had no effect on miniature IPSCs, implying an indirect mode of action. The effects of substance P were unaltered by NMDA-, $GABA_B$-, opioid-, adenosine A1- and $5HT_{1A}$ receptor antagonists, but were abolished by the broad-spectrum metabotropic glutamate receptor (mGluR) antagonist LY341495, the mGluR5-specific antagonist MPEP and the cannabinoid CB1 receptor-specific antagonist AM251. Consistent with this, substance P enhanced synaptic glutamate release, as revealed by an increase in the frequency of action potential-dependent spontaneous EPSCs. Finally, the effects of substance P on evoked IPSCs were mimicked and occluded by a glutamate transport inhibitor. **Conclusions:** Substance P may produce analgesia, in part, by enhancing glutamate-mediated excitation and endocannabinoid-mediated disinhibition of the PAG-RVM descending pathway.

POS-THU-078

MORPHOLOGICAL CHARACTERISATION OF EXTRINSIC INNERVATION OF MOUSE DISTAL COLON AND RECTUM

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Purpose: The extrinsic innervation of the mouse rectum and distal colon was studied using rapid anterograde tracing in vitro, with immunohistochemistry. Methods: Either the rectum or distal colon was taken from humanely killed C57BL6 mouse and opened into a flat sheet. Biotinamide was applied to either the pelvic nerves or colonic nerves for 5 hours in culture and later visualised with streptavidin-labelled fluorophores. The major neuroanatomical structures filled were varicose axons in the myenteric ganglia, circular muscle, submucosal plexus and intramural and extramural blood vessels. Results: In the rectum (n=6) a distinct class of specialised flattened branching endings was labelled in the submucosa and superficial circular muscle. Of 179 such endings, 93.1±3.1% were immunoreactive for calcitonin gene-related peptide (CGRP); none was immunoreactive for tyrosine hydroxylase (TH). In rectal myenteric ganglia, (n=6), 29.9±3.0% of all varicose fibre were immunoreactive for CGRP, none was immunoreative for TH. Filling in the distal colon revealed very different populations of extrinsic nerve endings. In myenteric ganglia, (n=5), 26.4±3.8% of varicosities were CGRP and 23.9±5.7% were immunoreactive for TH. In submucosal blood vessels, 33.5±7.8% of varicose fibres were CGRP positive and 41.3±2.7% were TH positive. No specialised CGRP-immunoreactive flattened endings were visible in the submucosa in the colon. In freshly fixed mouse rectum, coexistence of TrpV1 and CGRP was very high. In myenteric ganglia, 99.7±0.2%(n=4) of varicose fibres with Trpv1 also contained CGRP. Similarly on submucosal blood vessels (n=4) there was 99.0±0.7% coexistence. Conclusions: Both colon and rectum receive many peptidergic extrinsic afferents innervating blood vessels and enteric ganglia. A further population of specialised peptidergic submucosal afferents exist in the rectum but not colon.

POS-WED-079

HIGH-FAT DIET INCREASES SEROTONIN SIGNALLING IN RAT INTESTINE

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Purpose: A high-fat diet is associated with subtle changes in gastrointestinal function and in the levels of intestinal messengers secreted from neuroendocrine cells. Enterochromaffin (EC) cells containing serotonin (5-HT) do not directly detect ingested fat, but they may respond in a compensatory fashion to altered gut function during obesity. Our aim was to characterize the uptake and release of 5-HT in a rat model of diet-induced obesity. **Methods:** Electrochemical methods were used to measure peak and steady state (SS) 5-HT concentrations and fluoxetine (1μM) was used to block the serotonin reuptake transporter (SERT) in control (age-matched, chow-fed; 508±16g; n=6) and high-fat diet rats (HFD; 740±26g; n=11). The levels of message for tryptophan hydroxylase 1 (TPH1) and SERT were determined by quantitative PCR while EC cell numbers were determined by counting immunohistochemically labelled cells. Paired and unpaired data were compared with a one way ANOVA ($P<0.05$) using a Tukey-Kramer post-hoc test. **Results:** In control rat ileum, SS levels of 5-HT were 12.3±3.4μM and peak compression-evoked release was significantly higher at 22.3±4.2μM ($P<0.05$; n=6; each sampled from 3 positions on the mucosa). In HFD rats, the levels of 5-HT were significantly increased (SS: 23.8±3.4μM; peak: 66.8±13.4μM; $P<0.05$; n=11). In chow-fed rats, fluoxetine doubled peak and SS 5-HT release (180% of control; n=3), while in HFD rats there were no significant changes. Rundown for compression-evoked release was similar in both control (n=3) and HFD rats (n=4; $P>0.05$) with peak release reduced by ~50% during a second response. There was no change in TPH1 or SERT message but the numbers of EC cells/crypt-villus doubled. **Conclusions:** Our data predict that a high fat diet will be associated with increased 5-HT availability. As these changes are not driven by altered genetic control, further work on the physiological regulation of 5-HT availability is needed.

POS-THU-080

RELEASE OF SEROTONIN FROM HUMAN COLONIC MUCOSA

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Purpose: Serotonin (5-HT) containing enterochromaffin (EC) cells of the intestine detect chemical and mechanical stimuli in the lumen and respond by releasing 5-HT on to afferent nerve terminals. Our aim was to characterize the real-time release of 5-HT from the mucosa of human colonic surgical samples. **Methods:** Segments of sigmoid colon (and one of transverse colon) were obtained with consent from 3 male and 3 female patients (age: 60±4 yrs) during surgery for colon cancer (bleeding, no therapy). Real-time electrochemical methods using a carbon fibre electrode were used to determine 5-HT oxidation current. Peak and steady state (SS) 5-HT concentrations (calculated from the oxidation current at +300mV) were measured from 9 positions on mucosa (with the underlying layers removed). Paired and unpaired data were compared using student's t test. **Results:** Fresh specimens had peak compression-evoked 5-HT release of 37±14μM (time to peak: 0.3±0.2s) while SS levels were lower (15±6μM; $P<0.05$; n=4). Nine positions on the mucosal were tested but robust release was seen from only 5±1 positions (n=4). Repeated compression at the same point caused a significant reduction in the peak 5-HT release during the second response (37% of control; $P<0.05$; n=4) but not the SS levels (66% of control; $P>0.05$; n=4). SS levels were also not different when measured by gentle touch (9±4μM) versus compression (9±4μM; $P>0.05$; n=4). A further 2 specimens stored in the refrigerator (1-2d) showed low levels of 5-HT release (peak: 5±3μM and SS: 2±1μM; n=2). **Conclusions:** The release of 5-HT from the EC cells of the human colon can be accurately measured using electrochemical methods. Understanding how released 5-HT acts at afferent nerve terminals will contribute to our knowledge of human intestinal disease.

POS-WED-081

SELECTIVE CHANGES IN CEREBELLAR-CORTICAL PROCESSING FOLLOWING MOTOR TRAINING

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Purpose: to investigate the effect of varying stimulation rate and the effects of a repetitive typing task on the amplitude of somatosensory evoked potential (SEP) peaks related to cerebellar processing. Methods: SEPs (2000 sweep average) were recorded following median nerve stimulation at the wrist at frequencies of 2.47 Hz, 4.98 Hz, and 9.90 Hz from 12 subjects before and after a 20 minute repetitive typing task. Typing and error rate were recorded 2 minutes pre- and post-typing task. Effect of stimulation rate was analyzed with ANOVA followed by pairwise comparisons (paired t-tests) with Bonferroni corrections. Typing effects were analyzed by performing two-tailed paired t-tests. Results: Increasing stimulation frequency significantly decreased the N30 SEP peak ($p<0.02$). Both the 4.98 Hz 9.90 Hz rates were significantly smaller than 2.47 Hz ($p\leq0.01$). The N24 data significantly increased following the typing task for both 4.98 Hz and 2.47 Hz ($p\leq0.025$). In contrast, there was a highly significant decrease ($P<0.001$) in the N18 peak post typing at all frequencies. Typing rate increased ($P<0.001$) and error rate decreased ($p<0.05$) following the typing task. Conclusion: Our results suggest that the N24 SEP peak is best recorded at 4.98 Hz since the N30 drops off and no longer contaminates the N24 peak, making the N24 most visible and easier to measure, while still enabling changes due to repetitive activity to be measured. The decrease in N18 along with an increase in N24 with no change in N20 suggests that the intervention lead to reduced inhibition at the level of the cuneate nucleus/inferior olives and a selective increase in the afferent information reaching the cortex via the cerebellum.

POS-THU-082

USING PARALLEL AFFERENT CHANNELS TO IMPROVE TACTILE SPATIAL RESOLUTION

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Purpose: Kajimoto et al. (2002, Electronics and Communications in Japan, Part 2, Vol. 85, No. 6) have suggested that different electrode and current configurations may be able to selectively activate Merkel (SA), Meissner (RA) and Pacinian corpuscle (PC) receptor associated afferents. We have quantitatively evaluated this claim using mixed mode stimulation designed to recruit different classes of tactile afferents to determine two point discrimination on the back of the hand, using both mechanical and electrical stimuli. **Methods:** We used three types of stimuli designed to preferentially activate SA, RA and PC afferents, and presented these as single or two point stimuli in a two alternative forced choice protocol. Stimuli were delivered through two vibrotactile stimulators, or by isolated electrical stimulation with an array of 25 electrodes based on the protocol of Kajimoto et al. of (2002). **Results:** Subjects (n=28 electrical, n=24 mechanical) were equally able to make two point discrimination judgements with either mechanical or electrical stimulation. Mixing stimulus modes to recruit different classes of afferents did not provide improvement in discrimination over same mode stimulation for either electrical or mechanical stimulation. Some modes of electrical stimulation (such as RA-PC) were much less well discriminated ($p<0.001$) than other modes (such as PC-PC) which was not observed for the mechanical stimuli. **Conclusion:** There are differences between afferent activation modes of the electrical stimuli, but they can not be used to support a functional increase in spatial resolution on the back of the hand for a proposed navigational aid for the blind.

POS-WED-083

CHANGES IN THE PROPERTIES OF CUTANEOUS RECEPTORS DUE TO AGE

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Purpose: Tactile sensitivity in the hands declines as a function of age. However, it is not known if this is due to changes in the skin or in the receptor end organ of cutaneous low-threshold mechanoreceptors. **Methods:** Sensation at eight sites in the glabrous skin of both hands was tested in 60 subjects (20–79 years), 10 per decade, using calibrated von Frey filaments and two point discrimination. Changes in skin mechanical properties were tested using venous occlusion (~40 mmHg). Skin turgor, or the ability of the skin to return to its normal contour after deformation, was measured to assess the effect of fluid volume in the finger. Changes in receptor properties were assessed by cutaneous afferent microneurographic recordings. **Results:** Under all conditions there was a significant effect of age ($p<0.001$). Perceptual thresholds were higher on the dominant hand ($p=0.05$). During venous occlusion, thresholds were significantly higher for the older age groups on the dominant hand only ($p<0.001$), while the non-dominant hand and younger age groups showed no systematic pattern of changes. The turgor measures were significantly lower in the older age groups for both hands ($p<0.001$). Single receptor neural thresholds were 64% higher in older subjects. Two point discrimination showed significant effects of age, but no effect for venous occlusion or hand dominance. **Conclusions:** These results suggest that declines in tactile sensitivity due to age result from changes in both the nerve and the skin on the dominant hand, while reduced sensation in the non-dominant hand is more related to receptor properties than skin mechanics. These results provide age appropriate normative data for studies in patients with reduced sensation, such as stroke.

POS-THU-084

DIFFERENTIAL CONTRIBUTION OF TACTILE AFFERENTS TO ALLODYNIA AND ANALGESIA

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It is unclear whether allodynia, or for that matter analgesia, results from activation of a single class of tactile afferent fibre or the convergence of inputs from multiple classes. This lack of clarity is of particular concern in clinical conditions where the line between innocuous (tactile) and noxious (painful) sensation is blurred. **Methods:** Sustained muscle pain was induced by infusing (100-200 µl/min) hypertonic saline (5%) into tibialis anterior. Prior to, during and following muscle pain, cutaneous vibration (200 Hz, 200 µm, 30 s) was applied to the skin overlying the muscle. Pain ratings were recorded using a Visual Analog Scale. **Results:** Prior to muscle pain, all subjects described vibration as non-painful. During muscle pain (VAS 4-6), 8 subjects reported an increase (allodynia) and 9 reported a decrease (analgesia) in pain intensity during vibration. These vibration-induced changes were significant, reproducible over time and abated in parallel with the underlying muscle pain. In all cases, allodynia (n=8) was abolished by intradermal anaesthesia (2% lignocaine) and on 3 occasions revealed an underlying analgesia. These results suggest that allodynia is dependent upon the activation of sensory receptors located in the skin overlying tibialis anterior. Intradermal anaesthesia abolished vibration-induced analgesia in 3 subjects and had no effect or amplified analgesia in 3 subjects. These results suggest that analgesia is evoked by the activation of cutaneous and deep receptors. **Conclusions:** Activation of cutaneous non-nociceptive afferent fibres preferentially induces allodynia. Afferent fibres beneath the skin preferentially induce analgesia. These observations suggest that it is the central convergence, and subsequent integration of nociceptive and non-nociceptive inputs that determines the overall perception of muscle pain.

POS-WED-085

THE ASTEX®: A NEW DEVICE FOR ASSESSMENT OF TEXTURE DISCRIMINATION IN THE HAND

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Purpose: The ability to appreciate the surface texture of objects is important for successful grasping and manipulation of objects. In this study the clinimetric properties of the AsTex®, a new instrument developed to assess texture discrimination in the hand, were investigated. **Method:** Test-retest and inter-rater reliability, and age- and gender-stratified normative values for texture discrimination indices (TDIs) were established in neurologically normal participants aged between 18 and 80 years (N=142). Test-retest reliability was also examined in chronic stroke patients (N=22). **Results:** The AsTex® required less than 5 minutes to administer and had good clinical utility. Test-retest (ICC=0.98, 95% CI=0.97-0.99) and inter-rater reliability (ICC=0.81, 95% CI=0.73-0.87) were found to be excellent in neurologically normal participants. Test-retest reliability of the AsTex® in individuals with stroke was also excellent (ICC=0.86, 95%CI=0.68-0.94). The standard error of measurement was 0.14 mm. In neurologically normal participants there was a trend for TDIs to rise (indicating poorer sensory discrimination) after the fourth decade. Subjects aged 70 years or older had significantly higher TDIs than younger subjects ($p < 0.001$). **Conclusion:** The AsTex® is a reliable and clinically useful instrument for quantifying hand sensation capabilities. The age- and gender-stratified normative data will facilitate the interpretation of AsTex® assessment findings in clinical populations.

POS-THU-086

SPATIAL TACTILE ACUITY AND ANISOTROPY ON HUMAN LIPS

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On the human finger pad, spatial tactile acuity is greatest when an object is applied parallel to the dermal ridges. This directional specificity is termed anisotropy. **Purpose:** The aim of our study was to determine whether anisotropy exists on human lips, an area that is well-adapted to processing spatial information due to dense receptor innervation and a large area of representation in somatosensory cortex. **Methods:** Spatial tactile acuity was assessed in 9 healthy subjects (age 23±4 yrs) with a three-alternative, forced-choice grating orientation task. Circular probes with horizontal (parallel to the lip), vertical (perpendicular to the lip), or oblique (45 degrees to the right of vertical) grooves and ridges of equal width were applied to the midline of the upper or lower lip. Participants were asked to state the orientation of the grating whilst blindfolded. Each probe was applied 60 times, 20 times in each orientation. The percentage of correct responses was plotted as a function of the log of the gap width. Data were fitted with a four-parameter sigmoid function. We adopted 75% correct as the threshold estimate. **Results:** Across lips, the average threshold for the horizontal, vertical, and oblique orientations was 1.0±0.8 mm, 1.6±1.1 mm, and 2.4±0.8 mm, respectively. There was a significant main effect of orientation on threshold ($P<0.001$). Threshold was lowest for the horizontal orientation and highest for the oblique orientation ($P<0.038$). **Conclusions:** Our results demonstrate that anisotropy is present on human lips. The tactile anisotropy could be due to greater skin compliance in the horizontal direction, the size and shape of receptive fields, and/or orientation-selective neurones in somatosensory cortex.

POS-WED-087

A PRECONDITIONING NERVE LESION ATTENUATES MECHANICAL ALLODYNIA, BUT DOES NOT ALTER NEUROINFLAMMATION, IN A RAT MODEL OF NEUROPATHIC PAIN

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Purpose: A preconditioning stimulus can trigger a neuroprotective phenotype in the nervous system - a preconditioning nerve injury causes a significant increase in axonal regeneration, and cerebral preconditioning protects against subsequent ischemia. We examined whether a preconditioning nerve lesion affects neuropathic pain and neuroinflammation due to peripheral nerve injury. **Methods:** Rats received a preconditioning crush injury to one of the three terminal branches of the sciatic nerve (tibial, common peroneal, or sural nerve) or no crush injury (control), 7 days before partial ligation of the sciatic nerve (a model of neuropathic pain). Measurements of pain behaviors (n=6 rats per group) and immunohistochemistry (n=3 rats per group) for macrophages and T cells in L4/5 dorsal root ganglia (DRGs) and glial cells in L4/5 spinal segments were carried out. **Results:** A preconditioning lesion of the tibial nerve induced a long-term significant attenuation of both ligation-induced mechanical allodynia and thermal hyperalgesia. A preconditioning lesion of the common peroneal or sural nerve induced a smaller but significant short-term attenuation of mechanical allodynia. There was no difference in macrophage and T-cell infiltration into the DRGs, or in astrocyte and microglia activation in the spinal dorsal horns of these animals. **Conclusion:** Our results suggest that a preconditioning nerve lesion 1 week prior to partial sciatic nerve injury reduces mechanical allodynia in rats and that this conditioning-induced attenuation of pain is not dependent on neuroinflammation in DRGs and spinal cord.

POS-THU-088

SENSORY INNERVATION OF THE EXTERNAL GENITALIA OF FEMALE GUINEA-PIGS

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PURPOSE: Unexplained pain from the external genitalia (vulvar vestibulitis syndrome) affects 15% of women. However, surprisingly little is known about the sensory innervation of female external genitalia. Therefore, we examined the distribution and neurochemical profile of sensory neurons innervating the external genitalia of female guinea-pigs. METHODS: The pudendal region and lumbo-sacral spinal cord of young adult female guinea-pigs were prepared for multiple-labelling immunofluorescence following fixation in 2% formaldehyde / 0.5% picric acid. Sagittal sections through the whole pudendal region, including rectum, urethra, vagina and vulva, of 3 guinea-pigs were labelled for combinations of calcitonin gene-related peptide (CGRP), substance P (SP), neuron specific enolase (NSE) and vesicular glutamate transporter 1 or 2 (VGluT1, VGluT2). Spinal cords were labelled for CGRP and SP. In addition, DiI was applied to the pudendal nerve attached to the spinal cord and maintained in vitro for 3 days, followed by immunohistochemical labelling of dorsal root ganglia (DRG), RESULTS: Dermal papillae in the vulva and clitoris were densely innervated by large diameter NSE-immunoreactive (IR) endings. Many were VGlut1-IR, probably representing low-threshold mechanoreceptors. Fine varicose NSE-IR fibres penetrated almost to the surface of the epidermis. Papillae also contained varicose CGRP-IR fibres without SP-IR. Fibres with both CGRP-IR and SP-IR surrounded deeper dermal vasculature. DiI applied to the pudendal nerve labelled CGRP-IR DRG neurons without SP-IR. CGRP-IR terminals without SP-IR formed a prominent plexus in lamina IV of the dorsal horn. Within the internal genital tract, all CGRP-IR fibres were SP-IR. CONCLUSION: CGRP fibres in the pudendal nerve may represent a class of polymodal nociceptors and a potential unique source of genital pain.

POS-WED-089

VAGAL AND SPINAL AFFERENT NEURONS INNERVATING THE ADULT MOUSE JEJUNUM HAVE DIFFERENT STRUCTURAL AND NEUROCHEMICAL PHENOTYPES

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Purpose: Sensory information from the gut is conveyed to the CNS by vagal and spinal primary afferents originating from the nodose ganglia (NG) and dorsal root ganglia (DRG), respectively. We have recently reported that many DRG neurons innervating the jejunum are medium-sized and express transient receptor potential vanilloid type 1 (TRPV1), nitric oxide synthase (NOS), calcitonin-gene related peptide (CGRP). It is unknown if the NG neurons innervating the jejunum have similar characteristics. Methods: Sub-serosal injections (each 0.1 µL) of cholera toxin B (CTB) were made into the jejunum of anesthetized C57Bl/6 mice (ketamine 100 mg/kg, xylazine 10 mg/kg; n=16). Dye leakage was prevented by a thin application of cyanoacrylate glue over injection sites. Immunohistochemistry of CTB-labelled NG cells were processed with antibodies for TRPV1, CGRP, NOS and binding for isolectin B4 (IB4) seven days post-operative. Cross-sectional areas of nucleated cell profiles were assessed using Image J software (NIH). Results: Almost all CTB-labelled NG neurons were small- and medium-sized (98%; 144 of 146 cells). CTB-labelled NG neurons innervating the jejunum are significantly smaller than DRG neurons supplying the same region (median: 295 vs 540 µm^2 respectively; $p<0.01$, Mann-Whitney). Most CTB-labelled NG neurons bound IB4 (81%) but relatively few expressed TRPV1 (32%). Less than 20% of CTB-labelled IB4 neurons co-expressed TRPV1. NOS (0%) and CGRP (0%) expression was absent from all CTB-labelled NG neurons. Conclusions: Based on somal sizes, NG and DRG neurons innervating the jejunum are likely to differ in their electrophysiological properties. Additionally, terminals of these vagal neurons (IB4+/TRPV1-/NOS-/CGRP-) may also be distinguished from those of enteric and spinal neurons (IB4-/TRPV1+/NOS+/CGRP+) in the intestinal wall.

POS-WED-091

TRANSDUCTION BY GUT MECHANONOCICEPTORS

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High threshold, peptidergic mechanoreceptors, sensitive to capsaicin, can be activated by blunt glass probes; in a non-physiological manner. Purpose: we investigated physiological transduction by these endings. Methods: Extracellular recordings of extrinsic afferents with transduction sites on submucosal blood vessels in guinea pig small intestine, were studied in vitro. Results: Circumferential stretch by applying 5 - 15g loads (corresponding to 13 – 40 mmHg intraluminal pressure), reliably activated submucosal vascular afferents, as did von Frey hairs of 3mN or greater. These mechanonociceptors had significantly higher mechanical thresholds to both stimuli than specialised low threshold rectal or vagal mechanoreceptors. Muscle contraction evoked by carbachol (1-10µM) also caused firing, suggesting that these afferents respond to distortion, rather than length or tension. Benzamil (100µM) significantly reduced firing evoked by both von Frey hairs and by distension >90% (n=4, $p<0.05$). The Trp channel blocker, SKF 96,365 (50µM), reduced both distension and by von Frey hair-evoked firing by >80% (n=4, $p<0.05$). Gadolinium ions (Gd3+, 100µM), significantly reduced firing to distension (by >60%, $p<0.05$) but not firing evoked by von Frey hairs (n=5, $p>0.3$), suggesting that it acts indirectly via the muscle rather than on nerve endings [3]. 5-hydroxytryptamine (1-100µM) and ATP both evoked increased in firing rate in all submucous mechanonociceptors tested (n=5). Responses to distension and von Frey hairs persisted in 0mM [Ca2+] with raised [Mg2+] indicating that they do not require fast, exocytotic release of chemical mediators from other cells. Conclusions: intramural mechanonociceptors endings of the gut respond to a range of mechanical stimuli, probably via mechanosensitive ion channels on their endings and can be modulated by a range of chemical mediators.

POS-THU-090

SEROTONIN RELEASE FROM THE MUCOSA IS NOT REQUIRED FOR THE GENERATION AND PROPAGATION OF COLONIC MIGRATING MOTOR COMPLEXES IN RODENTS

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Purpose: The pacemaker and pattern generator underlying colonic migrating motor complexes (CMMCs) has not been identified, but is thought to involve release of serotonin (5-HT) from the mucosa. We have determined the role of serotonin release from the colonic epithelium in the generation and propagation of CMMCs in isolated whole preparations of mouse colon. Methods: Carbon fibre electrodes were used to record the dynamic release of 5-HT from a population of EC cells in the mid colon, whilst recordings were made of spontaneously propagating CMMCs from the proximal to distal colon using isometric mechanical recordings. Results Each CMMC contraction was temporally associated with a cyclical rise in 5-HT release from the epithelium. To test whether the CMMC pacemaker required release of 5-HT from EC cells, the mucosa, submucosa and submucosal plexus were dissected from the entire colon. Removal of these structures abolished all cyclical rises in 5-HT release from the mucosa, but did not prevent the cyclical generation of CMMCs. Specifically, it was found that removal of the mucosa, submucosa and submucosal plexus caused a slight, but insignificant decrease in CMMC rhythmicity (control interval: 1.5 ± 0.27min; c.f. after mucosal removal: 2.0 ± 0.3min; n=4: $P=0.26$), but no significant differences in CMMC amplitudes (control: 38.8 ± 11.1mN, c.f without mucosa: 44.9 ± 7.6mN; n=4: $P=0.66$), or half durations. Conclusions The pacemaker and pattern generator underlying the cyclical generation and propagation of CMMCs is located within the myenteric plexus, and does not require cyclical or basal release of serotonin, nor any other endogenous substances released from the colonic epithelium.

POS-THU-092

THE SPHINGOSINE 1-PHOSPHATE RECEPTOR 2 IN MURINE SENSORY DRG NEURONS

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Purpose: Bioactive lipids like sphingosine 1-phosphate (S1P) modulate the growth and pain transduction in sensory neurons, but neither the nociceptive specific expression pattern nor the signalling pathways of S1P receptors are known. The $S1P_2$ receptor subtype is known to be involved in the NGF dependent neurite extension but its presence and function within sensory nociceptive neurons has not been investigated. Methods: We used real-time quantitative RT-PCR, multiple labelling immunohistochemistry and Western Blots for the detection of $S1P_2$ and RhoA in murine DRG, spinal cord and in primary cultured DRG neurons. Results: We could detect the mRNA for all five S1P receptor subtypes in DRGs and spinal cord but only for three ($S1P_{1-3}$) in acutely dissociated DRG preparations (each n = 5). The $S1P_2$ receptor protein could be detected in situ and in vitro in neurofilament 200 (NF200) positive but isolectin B4 negative sensory neurons. Within the population of $S1P_2$+/Nf200+ neurons (20% of sensory neurons), 23% were also CGRP positive. The $S1P_2$ receptor is transported to the central and peripheral endings indicated by the detection of the protein within the sciatic nerve and in the spinal cord dorsal horn (n=5). The $S1P_2$ is known to activate RhoA. In primary culture DRG neurons, S1P application was followed by a massive increase in the amount of RhoA-GTP (n=5). This effect could not be mimicked by selective $S1P_1$ receptor activation. Conclusion: The $S1P_2$ receptor is present in a peptidergic nociceptive neurons and non-peptidergic myelinated sensory neurons. Activation of the receptor may be coupled to the RhoA signalling.

POS-WED-093

ANTIBODY-MEDIATED TARGETING OF NOCICEPTIVE NEURONS

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Purpose: Nociceptive neurons signal tissue damage from the periphery to the CNS. They consist of functionally different subpopulations of nociceptive sensory neurons situated within dorsal root ganglia. Although nociceptive neurons are important for the development of chronic pain, it is extremely difficult to address individual classes of nociceptors *in vivo* as well as under culture conditions. A subtype dependent modulation of nociceptor function would massively improve the therapeutic potential for the treatment of several pain disorders. **Methods:** We used a monoclonal antibody directed against the NGF receptor $p75^{NTR}$ (MLR2) to test if it is possible to directly target the subpopulation of peptidergic $p75^{NTR}$ expressing neurons. The antibody was characterised and coupled to either the fluorochrome Atto488 or to a GFP encoding plasmid via a DNA binding agent. Murine primary DRG cultures and lumbar DRG *in situ*, 1 and 2 days after i.p. application of the antibody were used. Transfected neurons were detected and neurochemically characterised by immunohistochemistry and subsequent confocal microscopical analysis. **Results:** Application of 3 µg/ml Atto488-coupled antibody into the culture medium led to the cell specific uptake of antibody indicated by the presence of intracellular Atto488 fluorescence in CGRP-positive peptidergic but not in isolectin B4 positive non-peptidergic murine primary DRG neurons (experiments n = 3). In addition i.p. application of the fluorochrome-coupled or the plasmid-coupled antibody led after 1 and 2 days to specific Atto488 fluorescence and GFP expression in CGRP-positive nociceptive DRG neurons (experiments n = 2). **Conclusion:** The $p75^{NTR}$ antibody MLR2 has great potential for the subtype specific modulation of nociceptor function.

POS-THU-094

TERMINALS OF GLUTAMATERGIC NOCICEPTORS FORM MICRODOMAINS IN LAMINA I OF THE MOUSE DORSAL HORN

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Purpose: In mice, a population of presumed nociceptors that do not contain any known neuropeptides and do not label with the lectin IB4 express the vesicular glutamate transporter VGluT2. These nociceptors are the only ones capable of releasing glutamate in the superficial dorsal horn since other nociceptors mostly lack detectable expression of vesicular glutamate transporters or synaptic vesicle proteins. We have used *in vitro* anterograde labelling of primary afferent fibres in combination with immunohistochemistry to distinguish terminals of VGluT2 immunoreactive (IR) nociceptors from intrinsic spinal cord neurons that express VGluT2. **Methods:** Spinal cords with attached lumbar dorsal roots were isolated from C57/Bl6 mice (n=3). Neurobiotin (1%) was applied to all six lumbar dorsal roots on one side of the spinal cord and to the L3 dorsal root on the contralateral side for 4 hours at 35°C. Spinal cord sections were immunolabelled for VGluT2 and CGRP. The distribution of labelled fibres was examined using high resolution confocal microscopy. **Results:** Neurobiotin labelled primary afferent fibres were distributed throughout the dorsal horn. Fibres with immunoreactivity to either VGluT2 or CGRP, but rarely both, were predominantly restricted to lamina I of the dorsal horn. Here, microdomains of CGRP-IR or VGluT2-IR fibres were apparent across the mediolateral extent of the dorsal horn. Most VGluT2-IR fibres in deeper laminae and all VGluT2-IR fibres in the lateral spinal nucleus did not arise from primary afferent neurons. **Conclusion:** VGluT2 expressing primary afferent fibres were predominantly restricted to lamina I of the superficial dorsal horn where discrete microdomains could form functional units distinct from adjacent nociceptors expressing CGRP.

POS-WED-095

PROPERTIES OF POST-SYNAPTIC DORSAL COLUMN (PSDC) NEURONS IN THE CERVICAL AND THORACIC SPINAL CORD

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Purpose: The spinal postsynaptic dorsal column (PSDC) pathway carries nociceptive information from peritoneal and pelvic organs but its role in relaying nociceptive information from thoracic viscera, particularly the oesophagus, remains unclear. The properties of cervical and thoracic PSDC neurons were therefore studied. **Methods:** Under anaesthesia, retrograde tracer was injected into the dorsal column nuclei in caudal medulla in rats. Animals were later reanaesthetized with α-chloralose/ketamine(i.v.). A latex balloon was repeatedly inflated in the oesophagus over 1 hour to induce visceral nociception or inserted but not inflated (sham). Activated neurons were detected using immunohistochemistry for Fos protein. Retrogradely labelled PSDC neurons were mapped and intracellularly injected with Lucifer Yellow to reveal their morphologies. **Results:** The majority of PSDC neurons outside the spinal enlargements were in lamina III-IV of cervical segments and showed morphologies consistent with previous studies in cat lumbar cord. Labelled neurons in laminae III-IV had significantly more dendritic spread in the dorsoventral dimension than those located in lamina V ($P< 0.01$, n= 5 cells/lamina) but no difference was seen in rostrocaudal or mediolateral dimensions, cell body area or diameter. Fos-immunoreactive neurons were observed in both the spinal cord and nucleus tractus solitarius (NTS) following noxious oesophageal stimulation (Fos-immunoreactive neurons/section significantly greater than in sham $P< 0.007$, n= 10 sections). Sections contained both Fos staining and retrograde labelling but no double labelled neurons were seen. **Conclusions:** Cervical PSDC neurons outside of the spinal enlargements in rat show distinct morphological features which vary systematically between laminae. They do not appear to be activated by nociceptive inputs from the oesophagus.

POS-THU-096

SINGLE UNIT RESPONSE PROPERTIES IN AUDITORY CORTEX OF RATS WITH UNILATERAL HEARING DAMAGE

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Sensory cortex is capable of undergoing reorganizational changes following alterations to its input. For example, inducing a frequency-specific hearing loss induces profound changes to the normal organisation of the auditory cortex. While these changes are well documented, and may represent the basis for recovery, we do not know if the functional properties of neurons within the reorganized region change. In this experiment we studied the responses of single units within the auditory cortex of rats with normal hearing and rats that had their hearing damaged by exposure to a loud narrow band noise stimulus (cf 8 kHz, 1/3 octave) for one hour. Five control and 11 treated rats were used in this study. The treated rats were classified as having high (5) or low (6) hearing loss, as determined by auditory brainstem response audiograms. Conventional tungsten in glass recording electrodes were used to measure the extracellular responses to monaural and binaurally presented tone pips. We characterised monaural and binaural response properties from a total of 180 single units. The sound pressure level thresholds at characteristic frequency did not vary between normal and sound-treated animals. Within the area of cortex affected by the noise-trauma we found an increase in excitation in response to both monaural and binaural stimulation. Monaurally, there were significantly more cells that responded to ipsilateral stimulation in the noise-exposed animals compared to the controls. Similarly, the proportion of binaurally responsive cells that exhibited ipsilateral inhibition was significantly lower in the noise-exposed animals. These results indicate that following noise trauma the auditory cortex is more excitable. The increased excitation may contribute to the perception of tinnitus following hearing loss.

POS-WED-097

SINGLE AND DUAL SITE STIMULATION IN THE VENTRAL COCHLEAR NUCLEUS USING A PENETRATING AUDITORY BRAINSTEM IMPLANT

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Purpose: A major limiting factor of the present auditory brainstem implant (ABI) is its inability to access the tonotopic organisation of the ventral cochlear nucleus (VCN) as it only stimulates the surface of the structure. Previously, we have shown that VCN stimulation of a single point within an isofrequency lamina is not always frequency specific and in some cases does not elicit a response in the central nucleus of the inferior colliculus (CIC). Therefore, we propose that the implant may require a greater number of penetrating electrodes in each lamina and that simultaneous stimulation of more than one location within each lamina might provide increased speech perception. **Methods:** In this study, we hypothesised that dual site stimulation in similar VCN isofrequency laminae would lower CIC thresholds, increase dynamic ranges and increase frequency specificity over single site stimulation. Data were recorded in response to tones from 58 VCN and 164 CIC multiunit clusters in the anaesthetised rat (n=7). Each individual VCN site as well as pairs of sites in similar isofrequency laminae (n=39) were stimulated while recording CIC responses. **Results:** CIC sites responded to dual site stimulation with significantly lower thresholds, higher dynamic ranges, and a higher degree of frequency specificity compared to single site stimulation (Repeated Measures ANOVA, $p < 0.01$). **Conclusion:** As frequency specificity, thresholds and dynamic ranges are all factors linked to speech perception, this method of dual site stimulation could result in improvements in speech perception if incorporated in an ABI stimulation strategy.

POS-THU-098

ADENOSINE RECEPTORS ALTER COCHLEAR RESPONSE TO NOISE

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Adenosine is a constitutive cell metabolite which may have a pivotal role in cochlear protection from oxidative stress. High affinity adenosine receptors (A_1, A_{2A} and A_3) are differently expressed in cochlear tissues. Here, we report the role of adenosine receptors in the cochlear response to noise stress in the rat. Selective adenosine receptor agonists were delivered onto the round window membrane (RWM) and compound action potentials (CAP), summating potentials (SP) or the Auditory Brainstem Responses (ABR) were used to measure the effect on cochlear function before and after noise exposure. Gene expression levels of adenosine receptors in the noise-exposed cochlea were studied using quantitative RT-PCR. Our results show that sound-evoked cochlear potentials (CAP and SP) are not affected by local administration of adenosine receptor agonists. Exposure to broad band noise presented at 110 dB SPL for 24 hours resulted in permanent threshold shift and up-regulation of A_1 receptors. ABRs were recorded at frequencies ranging from 4-28 kHz before and 6 hours after noise exposure. Adenosine and selective adenosine receptor agonists (CCPA, ADAC, CGS-21680 and Cl-IB-MECA) were applied to the RWM using gel foam. The follow-up ABRs 48 hours after the cessation of noise showed that administration of adenosine and A_1 adenosine receptor agonists (CCPA and ADAC) ameliorated hearing loss at higher frequencies. In contrast, A_3 adenosine receptor agonist Cl-IB-MECA aggravated noise-induced threshold shifts, suggesting that the activation of A_3 adenosine receptors may contribute to tissue damage. A_{2A} adenosine receptor agonist CGS-21680 did not affect cochlear recovery in the noise-exposed cochlea. This study pinpoints A_1 and A_3 adenosine receptors as attractive targets for pharmacological interventions to restore noise-induced cochlear injury.

POS-WED-099

STEM CELL THERAPY FOR NOISE-INDUCED HEARING LOSS IN MICE

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Purpose: The primary cause of acquired hearing loss is degeneration of the sensory cells responsible for hearing, the hair cells (HCs) in the cochlea. Here we investigate the potential of a novel source of adult stem cells to replace damaged hair cells and improve hearing levels. We have previously established a surgical procedure for stem cell injection into the cochleae of deafened mice, and automated the threshold detection for auditory brainstem response (ABR) to assess changes in hearing levels. **Methods:** Tongue stem cells were isolated from adult mice and injected into deafened adult mice (n=9) via a lateral wall cochleostomy. Changes in auditory function were assessed with ABRs evoked by click and pure tone (16 and 20 kHz) acoustic stimuli. ABRs were recorded before and four weeks after stem cell- or sham-injection. **Results:** Noise-induced hearing loss was established by acoustic trauma, which yielded a 25 dB or greater threshold shift in the ABR. A comparison of pre- and post-surgery ABRs showed a smaller threshold shift after stem cell injection than after sham injection for 16 kHz and 20 kHz tone stimuli (unpaired t-test; $p<0.05$) but not for click stimuli. **Conclusion:** The results suggest that tongue stem cells can improve hearing levels for tone stimuli after acoustic trauma. Further experiments will also assess the potential of human sensory stem cells to affect age-related hearing loss.

POS-THU-100

EFFERENT INNERVATION IS PRESENT TO ALL REGIONS OF THE AUDITORY BASILAR PAPILLA OF LIZARDS

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Hair cells of the inner ear of vertebrates are generally innervated by afferent neurones to transmit the sensory information to the brain as well as efferent neurones to receive feedback from the brainstem. The function of the efferent feedback system is poorly understood and may have changed during evolution as different vertebrate groups acquired sensitivity to airborne sound and extended their hearing ranges to higher frequencies. Lizards show a unique subdivision of their basilar papilla (homologous to the mammalian cochlea) into a low-frequency (<1 kHz) and a high-frequency (>1 kHz) region. Most interestingly, the high-frequency region was reported to have lost its efferent innervation (Miller, 1992), suggesting it was insignificant or even functionally detrimental at higher frequencies. **Purpose:** We re-examined the innervation to the basilar papilla of 5 species of Australian scincid lizards, using immunohistochemistry. **Methods:** Choline acetyltransferase (ChAT) was used as an efferent marker. SV2, a marker for synaptic vesicles, confirmed the synaptic identity of label. Wholemounts and cryosections of the cochlear duct were analyzed with a Zeiss LSM 510 Meta Confocal Microscope. **Results:** ChAT-immunoreactivity was observed along the whole length of the basilar papilla, including the regions which had previously been reported to be devoid of efferent terminals. ChAT labeling was consistent with the typical distribution of synapses on hair cells and extensively colocalised with SV2 immunoreactivity. **Conclusion:** Contrary to earlier descriptions, our findings suggest that efferent innervation is a general feature of the hair cells in the basilar papilla of lizards, irrespective of tonotopic location. This re-enforces the notion that efferent feedback control of hair cells is a fundamental and important property of all vertebrate hearing organs.

POS-WED-101

FLASH VEP IS REDUCED IN CHILDREN WHEN PRECEDED BY AN AUDIO-VISUAL STIMULUS

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Purpose. Perception gains significantly from multisensory integration (MSI). It is unknown whether children with cochlear implants (CI) have similar MSI mechanisms, or how they differ developmentally from normal-hearing children. This study aimed to establish the pattern of normal MSI development in children with good hearing, so that meaningful comparisons can be made with children with CI in our next study. **Methods.** An audio-visual illusion was presented to 13 children (aged 9-11) and 11 adults (aged 19-40). When a single flash is paired with a double beep, participants often report illusory perception of a double flash. Participants counted flashes, and 64 channel EEG was recorded. **Results.** The illusion was reported more often by children than adults. Unisensory (UNI) and multisensory (MULTI) difference waves were calculated depending on whether the second flash followed a uni- or multi-sensory stimulus. In children the UNI difference wave was significant from baseline at right parieto-occipital electrodes at 295 ms. No differences were found for the MULTI wave in children, and neither wave was significant in adults. **Conclusion.** In adults processing of the second flash was unaffected by the uni- or multi-sensory preceding stimulus. In children the UNI wave indicated increased activity at a late stage of processing. In the MULTI wave however, this extra activity was not apparent. In adults, parietal-occipital object-binding systems may be fast and automatic enough that processing of the 2nd flash is unaffected by the presence of either uni- or multi-sensory stimuli preceding. In children these systems may be immature. A uni-sensory stimulus may initiate binding processes (in the context of this task) that require more top-down attentional control to terminate.

POS-THU-102

NEW STRATEGIES FOR ELECTRICAL STIMULATION OF THE CENTRAL AUDITORY PATHWAY: CONVEYING BEHAVIOURALLY RELEVANT INFORMATION

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Purpose: The presently available auditory brainstem implant (ABI) is designed to stimulate the surface of the cochlear nucleus (CN), the first station in the central auditory pathway. Given that the normal processing of acoustic information in the CN is different from sound processing within the cochlea; several clinical studies have indicated the need for a novel stimulation strategy for the ABI. In this study we aim to determine whether auditory information through an ABI can convey meaningful information. **Methods:** Following surgical implantation of electrodes into the CN, rats underwent fear conditioning with the frequency of the conditioning tone set to one of the characteristic frequencies of the electrode sites. Electrical stimulation paradigms (50 Hz to 800 Hz; up to 40μA) were tested to examine which stimulation strategy best induced the fear response. This innovative approach will allow a direct test of the effectiveness of stimulation strategies to convey behaviourally relevant stimuli. **Results:** Fear response was initiated upon stimulation of sites within the CN. Frequency and intensity of stimulation were varied and single and two site stimulation combinations were tested. In animals tested, a fear response was initiated when sites were stimulated within an isofrequency lamina corresponding to the conditioned tone frequency. **Conclusion:** The results of these studies form a basis for a new penetrating ABI design providing insights into coding and stimulation strategies.

POS-WED-103

THE EFFECTS OF DEAFNESS AND CHRONIC ELECTRICAL STIMULATION ON THE SPATIAL AND TEMPORAL CHARACTERISTICS OF AUDITORY NEURONS

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Purpose: Multichannel cochlear implants electrically activate auditory neurons along the tonotopic gradient of the cochlea. Discrete populations of auditory neurons are preferentially activated by different intracochlear electrodes to provide frequency information. However, very little is known about the precision with which spatial and temporal information is provided or how extended periods of deafness and chronic intracochlear electrical stimulation (ICES) influence this information. **Methods:** Neonatal cats (n=9) were ototoxically deafened and at two months of age received a multichannel cochlear implant containing seven active intracochlear electrodes. Environmentally derived ICES was delivered chronically via a clinical stimulator (Nucleus CI24M, Cochlear™) and processor (Esprit 3G, Cochlear™) for a period of six months. Control cats (n=4) with normal hearing thresholds were acutely deafened at the time of the electrophysiological experiment. Single unit electrophysiological experiments were carried out to measure the spatial selectivity of auditory neurons in response to electrical stimulation (200Hz pulse trains) delivered on each intracochlear electrode in monopolar or bipolar configurations. The temporal characteristics of auditory neurons were examined by measuring responses to electrical stimulation of increasing pulse rate (maximum following rate). **Results and Conclusions:** Electrical stimulation of individual intracochlear electrodes produced selective activation of auditory neurons using both monopolar and bipolar configurations. Chronic ICES did not alter the extent of spatial selectivity across the auditory neurons sampled compared to control cochleae. Auditory neurons within chronic ICES cochleae were able to respond to stimulus pulse trains with 1:1 firing at pulse rates that were significantly greater than auditory neurons in control cochleae ($p<0.001$). These results indicate that auditory neurons are sensitive to both spatial and temporal cues evoked by electrical stimulation in long-term deafened cochleae that have received chronic ICES.

POS-THU-104

HISTOPATHOLOGIC EFFECTS OF CHRONIC COCHLEAR IMPLANT USE

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Purpose: Spiral ganglion neurons (SGNs) are the target neurons for cochlear implants; therapies designed to prevent their degeneration, or the degeneration of more central portions of the auditory pathway, would therefore be expected to result in improved clinical performance among cochlear implant patients. There is continued debate as to whether chronic electrical stimulation (ES) of SGNs, in ototoxically deafened animals, results in the rescue of SGNs *in vivo*. The present study examined the effects of chronic ES, via a commercially available cochlear implant, on the cochlea and cochlear nucleus of long-term deaf animals. **Methods:** Sixteen cats were neonatally deafened with daily subcutaneous injections of neomycin sulfate, resulting in a severe-profound sensorineural hearing loss. At eight weeks of age, eleven of these animals were implanted with an eight ring scala tympani electrode array and lead-wire assembly and received chronic ES, using a commercial cochlear implant system, for >16hr/day, 7 days/week over 8-12 months to reflect normal clinical usage. On completion of the stimulation program, histopathological examination of the cochleae and cochlear nuclei were performed on all deafened animals and an additional 3 age-matched normal hearing controls. **Results:** There was no significant difference in SGN density between the implanted left cochlea and the contralateral, unstimulated right cochlea in all turns ($P's > 0.05$). However, chronic ES resulted in a significant ($P < 0.01$) reduction in the shrinkage of the anteroventral cochlear nucleus (AVCN) normally associated with long-term deafness. **Conclusion:** Chronic ES, delivered from a commercially available cochlear implant, is able to prevent some of the atrophy in the AVCN caused by long-term deafness; however, it is unable to rescue SGNs in long-term deaf animals. More research into clinically viable techniques for the preservation of SGNs *in vivo* is required.

POS-WED-105

RETINAL DYSFUNCTION IN AN ANIMAL MODEL OF RETINOPATHY OF PREMATURITY

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Aim: Retinopathy of prematurity (ROP) is characterized by vascular, glial and neuronal pathology. Here, we evaluated retinal function in a rat model of ROP and assessed whether treatments targeting the renin-angiotensin system are beneficial. **Methods:** Newborn Sprague-Dawley rats were exposed to either 80% oxygen until postnatal day 11 and then room air until P18 (ROP) or room air for the entire duration (control). Control and ROP rats either received 1) no treatment (n=10-12), 2) AT1-receptor antagonist, valsartan (4-40 mg/kg/day intraperitoneal; n=10) or 3) prorenin receptor inhibitor (HRP; 0.1 mg/kg/day miniosmotic pump; n=10-12) from P11-P18. Retinal function was assessed by electroretinography (ERG). Vascular response was evaluated by counting blood vessel profiles. **Results:** Treatment with valsartan or HRP reduced retinal angiogenesis in ROP. Retinal function was significantly reduced in all rats with ROP. We observed a significant reduction in the amplitude of the rod photoreceptor response (PIII) in rats with ROP compared with controls. Rod post-receptoral responses, including the amplitude of the PII and oscillatory potentials, were also reduced in rats with ROP. Although treatment with the AT(1)-receptor antagonist, valsartan or the prorenin receptor inhibitor reduced angiogenesis, not all prevented deficits in retinal function. **Conclusion:** Our results indicate that exposure to high-oxygen during the early postnatal period leads to significant deficits in the function of both the rod and cone pathways. In addition, treatments that prevent pathological angiogenesis do not necessarily prevent retinal dysfunction. Further work is required to understand the underlying mechanisms for neural dysfunction during ROP.

POS-THU-106

OXYGEN TOXICITY: GENE EXPRESSION CHANGES IN THE HYPEROXIA-CHALLENGED MOUSE RETINA

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In the later stages of some retinal degeneration, including retinitis pigmentosa, increases in oxygen (hyperoxia) are toxic to the surviving photoreceptors, thereby contributing to the progression of photoreceptor degeneration [1]. **Purpose:** To investigate gene expression changes caused by hyperoxia in the C57Bl/6J mouse retina. **Methods:** Mice were placed in constant 75% oxygen for 0 (controls), 3, 7 or 14 days. The retinal RNA from 4 animals at each time point was extracted, purified and used on an Affymetrix GeneChip® 430 v2 to elucidate gene expression. Experiments were run in replicate and analysis of the expression changes performed using GCOS v1.4 (www.affymetrix.com) and GeneSpring (www.agilent.com). **Results:** Hierarchical clustering showed that the greatest divergence from control gene expression levels was evident at 14d exposure to hyperoxia, when it is known that the retina has began to degenerate. Further analysis of gene changes at 14d revealed genes involved in apoptosis and stress responses, in the removal of free radicals as well as genes implicated in a variety of retinal diseases. **Conclusion:** The shift in expression between control and 14d exposure to hyperoxia can be related to changes in the stability of photoreceptors, which appear unaffected by hyperoxia at control and 3d and have begun to degenerate by 14d. Since hyperoxia might be a feature of all photoreceptor degenerations, understanding of the cells' response to hyperoxia may be important in devising ways of stabilizing the degenerating retina. [1]Stone J, Maslim J, Valter-Kocsi K, Mervin K, Bowers F, Chu Y, et al. Progress in retinal and eye research. 1999 Nov;18(6):689-735.

POS-WED-107

THE P2X$_7$ RECEPTOR MODULATES NEURONAL FUNCTION IN THE MOUSE RETINA

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Purpose: The role of the P2X$_7$ receptor as a mediator of neurotoxicity and cell death has been well documented. However the neuronal function of the P2X$_7$ receptor under normal physiological conditions is unclear. We aimed to determine the role of the P2X$_7$ receptor in the retina using the electroretinogram (ERG) to assess neuronal function in P2X$_7$-knock out (P2X$_7$-KO) and C57B6/J wildtype (WT) mice. **Method:** ERGs were recorded, *in vivo*, under dark adapted conditions from anaesthetized P2X$_7$-KO (n=10) and WT (n=10) mice. Flash intensity ranged from -5.2 to 2.1 log cd/m^2. At the highest intensities two consecutive flashes (0.8 sec inter stimulus interval) were used to assess the rod and cone responses independently. Animals were euthanized and their eyes were collected for histology. **Results:** The rod photoreceptor response (a-wave; PIII) was similar in WT and P2X$_7$-KO mice. In contrast, the rod post-photoreceptor response (b-wave; PII) and the oscillatory potentials (OPs) were significantly larger in P2X$_7$-KO than in WT mice. In addition the kinetics of the rod PII response was faster in P2X$_7$-KO mice. When the cone ERG was assessed, the P2X$_7$-KO mice had larger post-photoreceptor responses than WT mice. Gross retinal morphology was similar in WT and P2X$_7$-KO mice. This suggests that activation of P2X$_7$ receptors in the inner retina inhibits output of second order neurons affecting both the rod and cone pathway response. **Conclusion:** In the normal retina, the P2X$_7$ receptor likely provides excitatory input to amacrine cells that shape the visual response pathways indicating a role for this receptor under physiological conditions.

POS-THU-108

CLASS 3 SEMAPHORIN EXPRESSION FOLLOWING OPTIC NERVE TRANSECTION IN ADULT RAT

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Purpose: Class 3 Semaphorins (Sema3s) are a group of secreted axon guidance molecules in vertebrates. They are expressed in rat visual system during development and in adulthood, and their expression alters following CNS injury. This experiment aimed to catalogue changes in mRNA levels of Sema3s and their receptors in rat visual pathways following optic nerve injury. **Methods:** The left optic nerve was transected in adult (8-10 weeks) Wistar rats. Animals were sacrificed 1d (n=8), 3d (n=6) or 14d (n=6) post injury (PO). Ipsilateral retinae and contralateral superior colliculi (SC) were dissected out, and control uninjured adult retinae (n=4) and SC (n=5) were also collected. mRNA expression levels for Sema3 ligands (Sema3A, Sema3B, Sema3C, Sema3E, Sema3F), primary receptors (Nrp1, Nrp2), and co-receptors (PlxnA1, PlxnA2, PlxnA3, PlxnA4, L1CAM) were measured by quantitative PCR and normalised against two housekeeping genes (PPIA and 18s). **Results:** In retinae, Sema3B, Sema3F and PlxnA3 mRNAs were elevated 3d and 14d PO ($p<0.05$). L1CAM expression was higher 3d PO ($p<0.05$) but PlxnA1 mRNA was reduced 1d PO ($p<0.05$). PlxnA4 mRNA showed a biphasic response – significantly lower than control at 1d and 14d PO ($p<0.05$). In the SC, Sema3A expression was undetectable after injury but Sema3B mRNA levels were increased at 14d PO ($p<0.05$). PlxnA2 mRNA was elevated at 1d, 3d and 14d PO ($p<0.05$), whereas PlxnA4 and L1CAM mRNAs were both lower 14d PO ($p<0.01$). **Conclusion:** There was a mixture of early, delayed and late changes in the retina and SC. Interestingly many genes did not change, including the main receptors Nrp1 and Nrp2. This study forms a basis for further analysis of Sema3s following injury to the rat visual system.

POS-WED-109

SORTILIN LOCALISATION IN THE RAT RETINA DURING EARLY POSTNATAL DEVELOPMENT

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Purpose: Sortilin, or neurotensin-3 receptor is part of apoptotic pathways through interactions with the low-affinity p75-neurotrophic receptor and certain pro-apoptotic neurotrophins. Neurotensin-like peptides have also been localised to mammalian retinas in subsets of amacrine and ganglion cells. Despite recent evidence for involvement of sortilin in embryonic apoptosis of retinal ganglion cells, a possible developmental role of sortilin during the later phase of apoptosis is unknown. To investigate this, we aimed to localise sortilin in rat retinas over a time course during the early postnatal period. **Methods:** Retinas from postnatal day (P)0, P2, P5, P7, P10, P12 (n=11) and adult rats (n=5) were fixed with 4 % paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) for 20 min and cryostat-sectioned the retinas for immunolabelling with sortilin antibodies combined with other retinal cell markers. **Results:** Choline acetyltransferase positive starburst amacrine cells showed transient sortilin-like immunoreactivity (-IR) at P7 and P10, suggesting a role for sortilin during this period when there is remodelling of the retinal networks as visual input is initiated. Approximately half the Brn-3 positive ganglion cells were sortilin-IR at all ages tested suggesting sortilin protein localisation in these neurons is not developmentally regulated during this time period. Horizontal cells marked by position and calbindin-IR had sortilin-IR somata until P12, but not in adult retinas. **Conclusions:** The localisation of sortilin in select types of retinal neurons is developmentally regulated during the early postnatal period. Further investigation is required to understand exact developmental roles of sortilin.

POS-THU-110

ANALYSIS OF NON-GANGLION RETINAL CELLS IN A MOUSE MODEL OF GLAUCOMA

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Purpose: To assess the functional and immunohistochemical effects of chronic elevation of intraocular pressure (IOP) upon retinal cells to determine whether the insult is specific to ganglion cells in this animal model of glaucoma. **Methods:** Ocular hypertension was induced by laser surgery of the episcleral veins and trabecular meshwork with an 810nm diode laser in C57/BL6J mice (n=60). IOP was measured on day 2, 5, 10, 20, 30, 40 and 60. Retinal function was assessed by scotopic electroretinography (ERG) and individual retinal cell types visualized by immunohistochemistry for glial fibrillary acidic protein, protein kinase Cα, calretinin and parvalbumin. **Results:** Forty-eight laser-treated eyes maintained an IOP of 22 mmHg or above. Elevated IOP significantly affected scotopic threshold ERG responses (derived from ganglion cells). A small but detectable effect was also observed on outer retinal-derived components. Conversely there was no effect upon the amplitude of the oscillatory potentials. Histological changes were also evident; in the control retina GFAP was localized to astrocytes. Five days of elevated IOP resulted in labelling of the Müller cells, which was maintained up to 60 days. PKCα immunoreactivity was limited to rod bipolar cells, and was unchanged after elevation of IOP. Calretinin was localized to amacrine and ganglion cells whilst parvalbumin was restricted to ganglion cells. There was an apparent progressive decrease in numbers of amacrine and ganglion cells expressing these calcium-binding proteins in response to elevated IOP. **Conclusion:** These data suggest that whilst chronic ocular hypertension affects multiple retinal cell types, the main impact is upon retinal ganglion cell function.

POS-WED-111

DIOLISTIC LABELING OF INNER RETINAL NEURONS IN MARINE FISH USING A HAND-HELD GENEGUN

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Marine fish retina represents a very intriguing model for the study of vertebrate retinal anatomy due to the diversity of visual adaptations presented by different fish species. However, a limit to the systematic utilization of this feature is the difficulty of effectively staining and differentiating fish retinal neurons, due to the poor availability of specific markers. Diolistic labeling is a method for introducing dyes into cells using biolistic techniques. The use of lipophilic carbocyanine dyes, combined with particle-mediated biolistic delivery using a hand-held genegun, allows non-toxic labeling of multiple cells in both living and fixed tissue. Here I present a modification of the DiOlistic labeling protocol to stain inner retinal neurons in marine fish retina. The protocol includes three major steps: (i) the preparation of the carbocyanine-coated tungsten particles; (ii) the preparation of fish retinal slices; (iii) biolistic delivery of the carriers into inner retinal cells. Ten damsel fish (*Chromis viridis*) were used to set up the protocol and an overall number of 90 isolated retinal neurons stained comprising bipolar, horizontal, Muller, amacrine and ganglion cells. The method proved rapid (less than on hour) and consistent in staining isolated inner retinal neurons and might represent an effective alternative to estimate the relative retinal density of diverse neuronal types. It also unlocks new possibilities for comparative anatomical studies in marine fish retina.

POS-THU-112

DOES THE RETINA SUBSCRIBE TO A "BROADBAND PROVIDER?"

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Purpose: The transmission of information in the nervous system has a limited "bandwidth." Nowhere is this more evident than in the connection from the eye to the brain. This connection is made up of the axons of retinal ganglion cells (RGCs) which transmit information regarding the visual world in the form of spikes. Thus, bandwidth is limited in large part by the rate at which RGCs can generate spikes. While some RGC types like alpha cells can spike at rates in excess of 250 spikes per second, other cell types can reach only 50 spikes per second. In both cases, however, the voltage gated sodium channels (VGSCs) involved in generating action potentials are only de-inactivated after more than 50ms. So, how do RGCs manage to spike at rates up to one every 4ms? Recently, it was demonstrated that expression of the VGSC Beta 4 (β4) subunit may underlie the ability of neurons to spike repetitively at high rates. **Methods:** We therefore set out to determine the expression, localization and physiology of β4 in the rat retina using RT-PCR, immunohistochemistry and whole-cell patch clamp electrophysiology. **Results:** Our data demonstrate that β4 is present in the retina, being first detected at P7, increasing into adulthood (n = 21). Immunostaining of retinal sections for β4 (n = 3) revealed a dense arrangement of short, intensely labeled processes in the optic fiber layer, characteristic of RGC initial segments. In addition, recordings of rat RGCs demonstrated resurgent sodium currents, characteristic of β4 expression, in 6 of 15 cells tested. **Conclusion:** These data demonstrate that some RGC types do subscribe to a broadband provider, in the form of the VGSC subunit β4.

POS-WED-113

MEASUREMENT OF ADULT VISUAL ACUITY AND THE MORPHOLOGICAL DEVELOPMENT OF THE SEAHORSE (HIPPOCAMPUS ABDOMINALIS) FOVEA

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Purpose: The aim of this study was to measure the visual acuity in the seahorse *Hippocampus abdominalis* and to analyze the morphological changes in the fovea during post hatch retinal development. **Methods:** Adult *H. abdominalis* were monocularly tested with a modified dynamic visual acuity stimulus- black floating dots with 100% contrast - of varying spatial frequencies (n=6) or binocularly in a rotating drum with black dots of varying spatial frequencies with 100% contrast (n=6). The central retinal morphology of six adult and three seahorses at each age (1 week, 1, 4 and 6 months post hatch) was analyzed. **Results:** The behaviorally measured visual acuity of 0.2-0.3 cycles per degree was the same with both methods. The 1 week and 1 month old seahorses had a rod free zone in the central retina, but lacked a foveal pit. The foveal pit was first observed at 4 months with an adult like fovea present at 6 months. **Conclusion:** *H. abdominalis*, an ambush predator, develops its fovea after hatching and experiencing the visual world. The limit of visual acuity in the adult is lower than calculated nyquist limits of the photoreceptors. These data lay the foundation for further comparisons involving another seahorse species *H. kuda* which is characterized by a rod free area centralis lacking a fovea.

POS-THU-114

S-ADENOSYLMETHIONINE IS NEUROPROTECTIVE IN THE RETINA FOLLOWING ACUTE ISCHEMIC INJURY

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Purpose: Although a large number of photoreceptors survive ischaemia-reperfusion, their function may be impaired. Phototransduction in the retina requires a number of S-adenosylmethionine (SAM)-dependent methylation reactions e.g. creatine synthesis, phosphatidylcholine synthesis and transducin methylation. These methylation reactions may be down-regulated after ischaemia. The purpose of this study was to determine whether administration of SAM after ischaemia could improve retinal function. **Methods:** Ischaemia was induced in adult rat eyes (n=48) by increasing the intraocular pressure to 110 mmHg for 60 minutes. Immediately after ischaemia, SAM (2ul) was injected intraocularly into the vitreous, followed by daily oral administration of SAM + vitamin B12 + folic acid for a further 5 or 10 days. Retinal function was assessed by scotopic flash electroretinography (ERG). Retinal injury was assessed by morphometric analysis of retinal sections. The expression of the creatine transporter (CRT) was determined by immunohistochemistry. **Results:** A significant reduction in the amplitudes of the ERG a-waves, b-waves and oscillatory potentials was induced by the ischaemic insult followed by 5 or 10 days of reperfusion. Post-ischaemic administration of SAM for 10 days significantly attenuated the ischaemia-induced retinal dysfunction. SAM did not alter any parameter of the ERG in control, non-ischaemic retinas. Morphometric analysis showed that SAM also ameliorated the ischaemia-induced retinal swelling. Furthermore, an apparent decrease in creatine transporter expression was observed following ischaemia. **Conclusions:** These data suggest that creatine synthesis and transport, as well as other methylation reactions maybe compromised by an ischaemic insult, contributing to retinal dysfunction and injury. SAM supplementation may provide an effective neuroprotective strategy.

POS-WED-115

PHOTOBIOMODULATION PROTECTS THE RETINA FROM LIGHT-INDUCED DAMAGE

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Purpose: To test the benefit of 670nm red light irradiation in preventing light induced retinal degeneration. **Methods**: Young adult Sprague-Dawley rats were treated with 670nm red light 5 times before (Pre-treatment), during (Mid-treatment) or after (Post-treatment) exposure to 24h 1000lux white light (LD). Eyes from each experimental and appropriate control groups (n=4 in all groups) were collected in two separate time points; 1 day after the last treatment or LD (detection of acute effects) and 7 days after LD (detection of accumulative effects). Tissues were immersion fixed in 4% paraformaldehyde, cryoprotected in 15% sucrose and sectioned at 12 microns. Hoechst and classic staining (H&E) were used to assess retinal histology and TdT-mediated dUTP nick end labelling (TUNEL) technique was used to detect apoptotic profiles of retinal cells. Immunohistochemisty was used to visualise structural and molecular changes in retinal tissue. **Results**: In control tissues, areas primarily affected were the RPE and outer retina. The damage was not uniform across the tissue with severe disorganisation in the superior half of the retina. This area was characterised by disruption of RPE, Bruch's membrane, infiltration of macrophages and massive reduction of the photoreceptor population. Tissues from animal groups treated with 670nm light showed significant reduction in photoreceptor cell death ($p<.0001$) and increase in photoreceptor survival ($p<.0001$). Integrity of retinal histology was preserved in all treated animals, most prominently in the Pre-treatment group. The expression of immune response markers was strong in controls, was present but significantly weaker in all treated groups. **Conclusion**: Photobiomodulation, treatment with a particular light wavelength, in this case the 670nm red light, could present a novel therapeutic alternative in treatment of some retinal degenerative conditions.

POS-THU-116

PHOTORECEPTOR AND GANGLION CELL TOPOGRAPHY IN THE PIGEON RETINA

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Purpose: This study aims to characterise the photoreceptor and ganglion cell densities and ratios across the adult pigeon retina and compares these findings with primate retinal topography. **METHODS:** Ten eyes from adult pigeons (*Columba livia*) were obtained post-mortem and fixed in 4% paraformaldehyde. Eyes were either flatmouned or cryosectioned. The flatmounted eyes were used for photoreceptor density counts and rod opsin immunocytochemistry. Frozen sections were stained with Propidium Iodide, labelled with rod opsin, and photoreceptor and ganglion cell densities determined. **RESULTS:** In the adult pigeon fovea was rod free with a peak photoreceptor and ganglion cell density of 326,000 and 110,000 cells/mm^2, respectively, yielding a 3:1 ratio. The area dorsalis peak photoreceptor and ganglion cell densities were 240,000 and 75,000 cells/mm^2, respectively yielding a ratio of 3.2:1. However there was twice as much variability in cell density in the area dorsalis compared with the fovea. Remaining regions (yellow field) had significantly lower photoreceptor (153,371 cells/mm^2) and ganglion cell (38,692 cells/mm^2) densities. The average rod photoreceptor density outside the fovea was 24,000 cells/mm^2. **CONCLUSION:** These data indicate that pigeon fovea is a rod free pit at the centre of gaze that contains the highest density of cone photoreceptors and ganglion cells. The area dorsalis is an additional area of high cone and ganglion cell density, although rod photoreceptors are present. These data show that pigeon fovea is a good model for the primate fovea in that it shares a number of important morphological characteristics.

POS-WED-117

TIME COURSE OF NEURONAL AND GLIAL CELL CHANGES IN THE RETINA DURING LIGHT-INDUCED RETINAL DEGENERATION

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Purpose: We were interested in determining the progression of retinal changes in a light-induced model of retinal degeneration. **Methods:** To induce retinal degeneration, 5 week old Sprague-Dawley rats were exposed to bright light (> 10000 lux; 16 hours) delivered via fluorescent tubing placed above the cage. Retinal function was assessed using the paired-flash protocol of the electroretinogram (ERG; n=10) prior to, and 2, 14 and 30 days following light exposure. For morphological analysis, posterior eyecups were fixed and processed for indirect fluorescence immunohistochemistry. Photoreceptor apoptosis was detected using the TUNEL method (Promega). **Results:** Immediately following light exposure there was a significant reduction in rod and cone photoreceptor function and an increase in apoptotic nuclei in the outer nuclear layer (ONL). At 14 and 30 days post light exposure, retinal function improved but remained reduced in comparison to control animals. Gliosis was apparent from 30 hours as shown by an increase in GFAP and Nestin expression. Microgliosis occurred as a response to dying photoreceptors from 2 days. The retinal pigment epithelium was absent in areas of photoreceptor loss at the 30 day time point. Involvement of purines in the glial and microglial response to light damage was observed; there was increased immunoreactivity to $P2Y_1$ receptors in Müller cells and microglia displayed altered P2Y receptor expression. **Conclusions:** Light damage is a useful model for examining functional and cellular changes occurring during retinal degeneration and also displays many of the hallmark features of age related macular degeneration. Further work is necessary to determine the role of purines in retinal degenerative disease.

POS-THU-118

SELF-REPAIR OF CONES IN THE RHODOPSIN-MUTANT RAT RETINA BY MANAGEMENT OF AMBIENT LIGHT

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Purpose: To assess the effect of ambient light levels on the integrity of cones in the rhodopsin-mutant P23H-3 rat retina, a model of autosomal dominant retinitis pigmentosa. **Methods:** P23H-3 rats were raised in scotopic cyclic (12 h at 5 lux, 12 h dark) ambient light. At adulthood, animals were transferred to photopic conditions (12 h 300 lux, 12 h dark) for 2-7 days, and then returned to scotopic conditions for 2-5 weeks (n=7 per group). Retinas were examined for cell death, for outer segment morphology using immunohistochemistry and electron microscopy, and for photoreceptor function using the electroretinogram. **Results:** Exposure to photopic ambient light for 2-7 days did not measurably reduce cone numbers, but caused shortening and disorganisation of cone outer segments, and a 40-60% reduction in the amplitude of the cone b-wave. Restoration of scotopic conditions for 2-5 weeks allowed recovery of the cone b-wave amplitude to 87% of control values, and regrowth and reorganisation of outer segments. **Conclusion:** The visual responsiveness and outer segment morphology of cones in the P23H-3 retina are rapidly and significantly reduced by a modest increase in ambient illumination. These changes are largely reversible if light levels are returned to scotopic conditions. This study demonstrates the capacity of cones to repair their structure and regain function and suggests that, in humans suffering photoreceptor dystrophies from comparable causes, the maintenance of steady, low ambient light conditions will optimise cone-based vision.

POS-WED-119

INVESTIGATION OF PUPIL RESPONSES TO MULTIFOCAL STIMULI SUBTENDING 10° AND 15° VISUAL FIELDS

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Purpose: To investigate contraction amplitudes of multifocal stimulus arrays subtending ±10° and ±15° of the visual field. **Methods:** Pupillary contraction amplitudes were analysed for 8 normal (mean age 24.1 ± 5.9 years) subjects with 6 different stimulus protocols extending to either ±10° or ±15° eccentricity. Stimuli were presented dichoptically and pupil responses were measured concurrently. A dart board layout having 24 or 44 independent test regions/eye with a mean presentation interval of 1, 2 or 4 s/region and a flicker rate of 30 Hz on each presentation was tested. Luminance of stimulus regions was 210 cd/m^2 and background 10 cd/m^2. Test duration was 4 minutes separated into 8 segments of 30 s recording intervals. Cameras under infrared illumination monitored pupil responses. Data during blinks and fixation losses were excluded to a maximum of 15% of responses beyond which a segment was repeated. **Results:** Multifocal stimuli extending across both ±10° and ±15° eccentricity resulted in larger responses for protocols with 4 s/region presentation rate and 24 regions/eye stimulus layout (b = 1.86 dB, t = 23.36, p <.00001) and (b = 3.63 dB, t = 42.67, p <.00001) respectively. The stimuli extending to ±15° eccentricity gave significantly larger responses than the ±10° stimuli by a factor of 1.6 (b = 2.06 dB, t = 29.30, p <.00001). **Conclusion:** Larger responses from the stimulation of broader retinal areas in normal subjects may be explained by a greater density of activated receptors producing a larger signal. This is an important factor in the pupillary evaluation of visual field defects by raising the areas of the field with reduced response sizes.

POS-THU-120

SLOWING VISION: PATTERN PULSE MULTIFOCAL VISUAL EVOKED POTENTIAL (PPMFVEP) TIMING DILATION UNDER ISOLUMINANT AND LUMINANCE CONTRAST CONDITIONS

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Purpose: The magnocellular (M) pathway's contributions to PPmfVEPs was examined by comparing VEP characteristics with isoluminant (ISO) colour and diffuse red background dartboards against Luminance Contrast (LC) dartboards. **Methods:** Participants (n=5) observed stimuli through a stereoscopic display (two screens viewed via mirrors at 45°; lenses gave an effective infinity viewing distance). Stimulus was an 84 region cortically scaled dartboard, eccentricity 23°. 4x4 checkerboards briefly pulsed pseudo-randomly (mean frequency 2/second/region). Ten conditions were tested with 30 or 60 cd/m^2 mean luminance and grey or chromatic (red-green) checks yielding six LC and four ISO conditions. RGB values were selected with a photometer. Participants viewed a 30Hz reversing dartboard of all regions; for ISO they made it not flicker; for LC they verified it flickered, they then viewed PP dartboards (four 1 minute segments per condition). 64 channels were recorded at 256Hz. **Results:** ISO produced a response delayed in initial rise and in peak relative to LC reflecting the absence of faster M pathway transmission for ISO. 90% of the channels were significant (p<0.03), with over 50 times power to signal to noise ratio (SNR) for occipital channels. Interestingly, the response to isoluminant stimulus is also larger in amplitude, despite the M component's absence. **Conclusions:** PP presentation has better SNR than traditional contrast reversing stimuli, allowing for more conditions in a reasonable length session. This research is the first usage of PPmfVEPs to identify M pathway effects. Potential applications include Dyslexia and Schizophrenia, which have a correspondence with dysfunctional M pathways.

POS-WED-121

WIDESPREAD, STIMULUS-INDEPENDENT CORRELATIONS BETWEEN NEURONS IN THE LATERAL GENICULATE NUCLEUS OF ANAESTHETISED MARMOSETS

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Purpose: We previously demonstrated stimulus-independent correlations between neurons in the dorsal lateral geniculate nucleus (LGN) of marmoset [1]. Here we asked whether such correlations are localised or widespread. **Methods:** Extracellular responses were measured in sufentanil-anaesthetised marmosets (n=9) using single electrodes or tetrodes. Receptive fields were characterized using drifting gratings. Responses to these stimuli and to 0% contrast ("spontaneous activity") were measured. Cell-cell correlations were estimated from Z-transformed average firing rates across 2-3 second trials spread over several minutes. **Results:** Significant correlations ($p<0.01$, Pearson statistic) were more common between magnocellular cell pairs (MC-MC; 22/61, 36%) than between parvocellular cell pairs (PC-PC; 9/44, 20%). Most pairs with significant correlations were positively correlated (n = 30, mean: 0.47, range 0.81 to 0.22). One pair was negatively correlated (-0.42). Correlation strength did not differ with respect to cell polarity (On– or Off-centre), or receptive field overlap. Correlations were also present in cell pairs that received inputs from opposite eyes (11/37, 30%). This suggests that the correlations could reflect an extra-retinal source having a widespread influence in the LGN. Correlated activity tended to be periodic with a long time constant (>60 s). **Conclusions:** Stimulus independent correlations are a consistent feature of responses in LGN neurones of anaesthetised marmosets. These correlations likely reflect slowly-modulated inputs affecting large regions of the LGN. [1] Cheong et al. (2008) Proc. Aust. Neurosci. Soc. 8, p60.

POS-THU-122

BRIEF EXPOSURE TO HIGH CONTRAST REDUCES THE CONTRAST SENSITIVITY OF MAGNOCELLULAR CELLS IN THE PRIMATE LATERAL GENICULATE NUCLEUS

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Purpose. Prolonged exposure to an effective visual stimulus leads to a reduction in the contrast sensitivity of magnocellular (M) and parvocellular (P) cells in the primate lateral geniculate nucleus (LGN). We asked if similar reductions were seen after brief exposures. **Methods.** Extracellular single-unit recordings were made from the LGN of 6 anaesthetized adult male marmosets (*Callithrix jacchus*). Contrast response was measured for short (0.2 s) presentations of a drifting grating of optimal configuration for the cell under study, after 0.5 s adaptation to a high contrast grating, or after a blank screen held at the mean luminance. The adaptor either drifted at the preferred rate, or was static and presented in either the preferred or anti-preferred spatial phase. **Results.** M-cells (n = 37): drifting adaptors reduced contrast sensitivity (impulses/s/[unit contrast]) by 23% (SD 26; $p < 0.01$, signed rank test). This reduction was more pronounced when the test stimulus was presented 0.1 s after the end of the adaptor than when that gap was 0.2 or 0.4 s, but contrast sensitivity remained depressed at these longer timescales. Static adaptors presented in either preferred or anti-preferred phase did not reduce the contrast sensitivity of M-cells (in both cases $p > 0.1$; n = 13). P-cells (n = 7): brief, drifting adaptors did not reduce contrast sensitivity (it increased by 12%, SD 15; $p = 0.05$). **Conclusion.** Contrast adaptation in the LGN can be induced within the normal duration of fixations, and might be important for optimising the operational range of M-cells during natural viewing.

POS-WED-123

COMPARATIVE ANALYSIS OF THE PRIMARY VISUAL CORTEX (V1) NEURONAL POPULATIONS IN CRX-KNOCKOUT AND WILDTYPE MICE

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Purpose: The transcription factor Crx (cone-rod homeobox) has a pivotal role in the morphological differentiation of both rod and cone photoreceptors in the retina. Targetted mutation of mouse *Crx* results in failure of growth of the outer segment of the photoreceptors and thus ganglion cells lack any visually-evoked activity. Mutation of the human *Crx* gene results in the inherited retinal blindness disease, Laber Congenital Amaurosis, which exhibits complete or near complete absence of vision from birth. **Methods:** In this study we examined the neuronal profile and function of specfic subpopulations of neurones in the primary visual cortex (V1) by employing antibodies against nonphosphorylated neurofilament (NNF), calbindin, parvalbumin and Fos in *Crx*-knockout (*Crx*-/-; n=10) compared to wildtype mice (C57BL/6; n=6). **Results:** In *Crx*-/- mice there was no difference in the total number of NeuN-immunopositive neurones observed in V1, when compared to wildtype controls. However, there was a significant decrease in both parvalbumin (65% in all layers) and calbindin (55% in layers 2/ 3) expressing neuronal subtypes, as well as a decreased amount of neuropil staining of the cells that remained immunopositive. The dense distribution of NNF-immunoreactive neurones and neuropil was decreased in layers 4&5. Following photic stimulation, there was also a reduced expression of Fos in layer 4 compared with the wildtype controls. **Conclusion:** This study demonstrates that an absence of normal retinal activity during development does not interfere with the structural development or the lamination of V1. However, the biochemical profile of the neurones is altered, and activity of these cells is significantly altered.

POS-THU-124

BYPASSING V1: DEVELOPMENT OF DIRECT RETINOTHALAMIC INPUTS TO THE MIDDLE TEMPORAL (MT) VISUAL CORTICAL AREA

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Purpose: In this study we describe the connectional development of the primate retino-pulvino/ geniculo-MT pathways and morphological development of the pulvinar, dorsal lateral geniculate nucleus (LGN) and MT areas. **Methods:** A combination of tracers and immunohistochemistry were used to identify pathways and demarcate areas in marmoset monkeys (*Callithrix jacchus*) aged from embryonic day 130 to adult (n=12). Photomicrographs of adjacent sections expressing nonphosphorylated neurofilament, calbindin and parvalbumin were interleaved with digitised images of fluorescent labelling and imported into a suite of programs called IMOD from which 3D models could then be constructed for connectional, morphometric and volumetric analysis. **Results:** In the medial nucleus of the pulvinar (PIm) we observed dense labelling of relay cells to ipsilateral MT area. In the adult a small number of these cells were recipient of retinal projections predominately from the contralateral eye whereas younger animals received increased labelling of binocular input to the PIm. At the level of the LGN, a number of labelled relay cells to area MT were observed. In younger animals these cells were retinotopically organised throughout all layers of the LGN and become restricted to the koniocellular layers in the adult. The volume of the pulvinar and PIm changes throughout development to reach adult values of 5.09mm^3, and 0.472 mm^3, respectively. **Conclusion:** This study provides evidence of the development of nonstriatal pathways from the retina to area MT early in life, prior to the maturation of all visual cortical areas. Furthermore, this demonstrates that these pathways could be, in part, responsible for the residual sparing of vision following a lesion of V1 observed in neonatal primates and humans.

POS-WED-125

SILENT SUPRESSIVE SURROUNDS OF NEURONES IN CAT AREA V2

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The receptive fields of most neurones recorded from cytoarchitectonic areas 17 (area V1) of carnivores or primates contain 'silent' so-called extra-classical receptive fields (ECRFs). Although the stimulation of ECRF with appropriate visual stimuli does not result in generation of action potentials, it significantly affects (usually reduces) the magnitude of response of the classical receptive field (CRF). Overall, the ECRFs of V1 neurones appear to contribute strongly to 'contextual modulation' of spike-responses. **Purpose:** To examine the properties of ECRFs of neurones in one of the 'higher-order' visual cortical areas. **Methods**: We recorded the spike-responses of single neurones located in cytoarchitectonic area 18 (area V2) of anaesthetized domestic cats. We compared the spike-responses to optimised patches of achromatic, drifting sine-wave gratings restricted to the CRFs with those generated when the gratings extended into the ECRFs. **Results:** Although at any given eccentricity the CRFs of V2 neurones are 2-3 times larger than these of V1 neurones, the proportion of V2 neurones with strongly (>50%) suppressive ECRFs at ~ 75% (37/48) was greater than that (~50%) in area V1 (Bardy et al., 2006; *J. Physiol*. 574.3:731-750). The spatial frequencies resulting in the greatest suppression were virtually the same as these producing strongest responses to the CRF-restricted stimuli. Furthermore, as in ECRFs of V1 neurones, most effective suppressive orientations were in most cells the same or similar (± 45 deg) as orientations which produced the strongest responses to CRF-restricted stimuli. **Conclusions:** Suppressive ECRFs of cat V2 neurones appear to contribute strongly, and in a stimulus specific manner to contextual modulation of spike-responses.

POS-THU-126

VISUAL PATHWAYS TO THE PRIMATE DORSOLATERAL PREFRONTAL CORTEX: A RETROGRADE TRACING STUDY IN THE MARMOSET MONKEY

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Having previously defined the subdivisions of the New World monkey frontal cortex (J Comp Neurol 495:149, 2006), we now report on the complete pattern of extrastriate afferents to this region, with the aim of defining frontal areas likely to be involved in visual cognition. Retrograde tracer injections were placed in frontal cortex of 4 marmoset monkeys under steroid (Alfaxan) anaesthesia. Two weeks later the animals were euthanased by barbiturate overdose, and their brains processed to visualise the locations of injection sites and labelled neurones relative to architectural fields. We found that injections centred on the frontal eyefield region {ventral subdivision of cytoarchitectural field 8A (8Av) and adjacent field 12/45}, revealed the most extensive visual projections. Although the densest projections originated in areas V4 and MT, labelled cells were present throughout low-order ventral and dorsal stream areas (from V2, caudally, to MST, FSTd, and LIP, rostrally). Frontal spatial working memory area 46 also received extensive projections from visual cortex, which originated primarily from higher-order subdivisions (TE, posterior parietal and caudal cingulate areas). Frontopolar area 10 received visual connections predominantly from high-order ventral stream cortex (area TE), a region concerned with face/object recognition, while area 9 received visual input predominantly from high-order spatial cognition areas (caudal cingulate, retrosplenial parahippocampal and superior temporal areas) and FSTv. Area 10 also received minor inputs from these regions. Finally, injections in other subdivisions of the caudal prefrontal cortex (cytoarchitectural fields 8A dorsal and 8B) resulted in no labelled cells in visual cortex. These results demonstrate extensive direct "shortcuts" that may enable different hierarchical levels of visual cortex to influence spatial cognition and other decision-making behaviours.

POS-WED-127

DIRECTION AND CONTRAST TUNING OF MACAQUE MST NEURONS DURING SACCADES

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Saccades are rapid eye movements that change the direction of gaze, however the full-field image motion associated with these movements is rarely perceived. The attenuation of visual perception during saccades is referred to as saccadic suppression. The mechanisms that produce saccadic suppression are not well understood. **Purpose:** We provide evidence for a neural correlate of saccadic suppression and expand on two contentious results from previous studies. First, we confirm the finding that some neurons in MST reverse their preferred direction during saccades. Second, it had been noted that neural activity associated with saccades can arrive in the parietal cortex up to 50ms sooner than visual activity produced during fixation, leading to the question whether the saccade related responses were visual in origin, or were motor signals arising from saccade-planning areas of the brain. **Methods:** We recorded from 79 neurons in the dorsal medial superior temporal area (MST) of three alert macaque monkeys and compared the neural responses produced by the retinal slip associated with saccades to responses evoked by identical motion presented during fixation. **Results:** We confirm that 30% of MST neurons reverse their directional tuning during saccades. By altering the contrast of the textured background in the saccade task we show that ultra-short latency saccade responses were visual in origin. We show that saccadic suppression is relatively stronger at low contrasts, suggesting the involvement of a gain control mechanism. **Conclusions:** Refinements of the possible models of saccadic suppression, involving short-latency alternate retino-cortical pathways will be presented.

POS-THU-128

SACCADIC SUPPRESSION AND POST-SACCADIC ENHANCEMENT OF VISUAL RESPONSES: TIME COURSE AND MECHANISMS

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Purpose: Primates use saccadic eye movements to make gaze changes. Visual sensitivity is reduced before saccades and enhanced afterwards. Here we characterise the time course of saccade related modulation of the underlying neural responses to visual stimuli. **Methods**: We recorded neural activity from 67 neurons in the dorsal medial superior temporal area (MSTd) in three monkeys (Macaca mulatta). Monkeys made rewarded saccades back-and-forth between two alternatively presented fixation points separated by 10°. Visual stimuli consisting of full-field random texture patterns were briefly presented at random intervals relative to the saccades. **Results**: Response amplitudes for flashes presented up to 90 ms before and during saccades were briefly suppressed by an average of 81% compared to controls. Immediately following this suppressive phase there was a period (up to 450 ms) in which response amplitudes were enhanced (mean peak enhancement 310%). Spontaneous activity was not suppressed for saccades made in the absence of visual stimuli. However, there was a consistent post-saccadic enhancement of spontaneous activity peaking 50 ms after saccade-onset. **Conclusion**: Spiking activity undergoes a stereotypical pattern of modulation around the time of saccades. As a whole, our observations suggest that (1) suppression of visual responses is inherited from earlier visual areas, (2) saccadic suppression is of central as opposed to retinal origin, and (3) post-saccadic enhancement appears to be, at least in part, of non-visual origin.

POS-WED-129

ATTENTIONAL BLINK IN THE MACAQUE: PSYCHOPHYSICS AND ELECTROPHYSIOLOGY

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Purpose: The attentional blink (AB) is a robust psychophysical phenomenon found in rapid serial visual presentation (RSVP), where detection of a second target is impaired when it is presented approximately 200-400 ms after an earlier target. We investigated whether the behaving macaque exhibits a similar phenomenon and whether a neural correlate exists in the lateral intraparietal area (LIP) within the posterior parietal cortex, which is believed to control attentional priorities. **Methods:** The monkey was trained on a delayed match to sample memory task, where he had to match both the location and orientation of two successively presented grating patches. Recordings were made from the LIP during the performance of the task. **Results:** We found that variation of the delay between the two gratings led to a function similar to the classical AB. When the delay was short and the second grating fell at the same location as the first, the error rates were high (around 40%). The performance reached near perfect scores (ca. 90%) at delays of 450 msec (ANOVA, $F = 6.518$, $p < 0.01$). The responses of some LIP cells also varied as a function of the delay between the first and second gratings, consistent with the behavioural data. **Conclusions:** Though imaging studies have implicated several cortical sites in the AB in humans, it has not yet been examined at a single-cell level in non-human primates. Our results provide the first single neuron correlates of an attentional overload similar to attentional blink in humans.

POS-THU-130

A MULTIFOCAL PUPILLOGRAPHIC INVESTIGATION OF DIRECT AND CONSENSUAL PUPIL RESPONSES

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Purpose: To investigate topographic variation in contraction amplitudes of direct and consensual pupil responses to multifocal stimuli. **Methods:** Pupillary contraction amplitudes were analysed from five studies undertaken over 15 months in which 120 normal subjects (mean age 54.0±13.7 years) were tested with differing subsets of 26 stimulus protocols. All stimuli were dichoptically presented and responses of both pupils were recorded concurrently. In the 26 stimulus protocols the multifocal stimulus arrays subtended ±30° of visual field and varied in: the number of stimulus regions (24, 40, 44 or 60), mean regional presentation interval (0.25, 0.5, 1, 4 or 16 s), and pulse time-course (33 to 150 ms flickered or steady). Luminance of the test-regions was 290 cd/m^2, background 10 cd/m^2. For each protocol the ratios between direct and consensual responses were calculated for each region. **Results:** Independent of regional differences in sensitivity, direct responses were significantly larger than consensual responses in the temporal hemifields for all 26 stimulus protocols. Across the 26 protocols the direct versus consensual differences ranged between 9.2% (b = 0.38 dB, t (1496) = 2.46, p <.02) and 34.0% (b = 1.27 dB, t (1528) = 4.44, p <.00001). These response differences were uniform across all temporal regions, and did not correlate with the median regional variations in response sizes believed to be due to differences in local retinal sensitivity. The magnitude of temporal differences for each protocol was somewhat correlated with the mean size of pupil contractions for the particular protocols. **Conclusion:** Stimulating the nasal retina produces larger direct than consensual responses in a pattern that does not correlate with the pattern of (afferent) retinal sensitivity. Uneven distribution of signals in efferent pupillary pathways is the likely cause.

POS-WED-131

DEMARCATION OF THE FERRET VISUAL CORTEX USING SPECIFIC NEURONAL IMMUNOLABELLING

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Purpose: The ferret is a commonly used model in visual cortical physiology and plasticity. The boundaries of its cortical areas have previously been characterized using electrophysiological and architectonic observations; however, there has been much dispute as to the exact location of its areal boundaries. In this study, we provide a new mapping according to the distribution of specific neuronal markers previously used to demarcate cortical area in other species by our group. **Methods:** Coronal and sagittal sections from the brains of adult ferrets (*Mustela putorius*; n=6) were processed with antibodies against nonphosphorylated neurofilament (NNF) and the chondroitin sulfate proteoglycan (CAT301). Expression profiles were subsequently plotted and areal reconstructions generated and compared with traditional histological staining profiles (e.g. Nissl). **Results:** In the adult ferret visual cortex, NNF and CAT301 immunoreactivity was distributed in a restricted laminar pattern among the individual areas. NNF immunolabelled neuropil was observed in layer 6 and 3 of areas 17 and 18, while both areas 18 and 19 had large cell body labelling in layer 5. The intensity of this labelling was also increased in area 19. With CAT301, changes in the intensity of neuropil immunolabelling in layer 3 were seen between areas 17 to area 21, to allow the clear demarcation of their boundaries. **Conclusion:** This study demonstrates that the specific neuronal antibodies NNF and CAT301 are useful in the accurate delimitation of the areal boundaries of the ferret visual cortex, and provide a more accurate and clearer identification than compared with the traditional histological stains.

POS-THU-132

EVOKED ACTIVITY DURING MULTISENSORY INTEGRATION IN CHILDREN AND ADULTS

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Purpose: Multisensory integration has a facilitating effect on information processing in adults. As the development of multisensory facilitation in children has not been previously reported, this study compared multisensory processing in children and young adults with normal hearing. **Methods:** Motor reaction times (MRTs), accuracy and high-density electroencephalography (64 scalp electrodes, sampling rate: 500 Hz, filter bandpass: 0.1 – 100 Hz at 12 dB/Oct) were recorded while children (N = 20) and adults (N = 14) performed a detection task. Stimuli included auditory (AT), visual (VT) and audiovisual (ATVT) targets presented for 40 ms. **Results:** Both adults and children were faster at detecting the ATVT stimulus compared to AT and VT stimuli. Cumulative density functions (CDFs) of MRTs showed that discrepancies between ATVT CDFs and AT+VT CDFs were greater for adults than children. Multisensory integration at a neural level was isolated by subtracting the sum of the two unisensory evoked potentials from the multisensory evoked potential (ATVT–[AT+VT]). Multisensory integration was observed 100 ms post stimulus onset at parietal electrode sites for children and occipital electrode sites for adults. After 150 ms post stimulus onset less integrative processes were observed in children than adults. **Conclusion:** Neural processes during multisensory integration have a different topographic distribution in children compared with adults. Both behavioural and electrophysiological measures suggest that multisensory integration is still immature in late childhood.

POS-WED-133

FOCAL ACTIVATION OF THE FELINE RETINA VIA A SUPRACHOROIDAL ELECTRODE ARRAY

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Purpose: Electronic retinal prostheses aim to elicit visual sensations to those blinded by diseases such as retinitis pigmentosa. Our group has developed a neurostimulator with the ability to electrically stimulate multiple channels simultaneously, driving electrodes in a hexagonal arrangement where each stimulating electrode is surrounded by six return electrodes (providing charge containment). We investigated the localisation of cortical responses at V1 through different electrode configurations of this novel hexagonal grouping of electrodes by electrically stimulating the retina from the supra-choroidal space of anaesthetised cats. **Methods:** A two-hexagon (14 channel) array of stimulating electrodes was inserted into the supra-choroidal space of anaesthetised cats (n=5). Charge balanced biphasic, bipolar stimulation was applied through the centre electrode from each hexagon group. Combinations of the surrounding six electrodes from each hexagon were used as returns: all six surrounds (hex-return), two neighbouring surrounds (two-return), or just one (single-return). Monopolar stimulation using a distant return electrode was also investigated. A 32 channel planar electrode array was placed on the cortical surface of V1 to record electrically evoked potentials. **Results:** We successfully elicited visual responses with average charge threshold for the hex-return strategy at 78.15±31.23nC. In terms of magnitude and area, the hex-return stimulation evoked responses 2.18±0.19 times smaller than the single-return stimulation ($P<0.0001$), and 1.89±0.18 times smaller than the two-return stimulation ($P<0.0001$). A greater response was also elicited for monopolar stimulation compared to bipolar stimulation. **Conclusion:** This supports the thesis that a hex-return bipolar stimulation strategy would evoke a more focal visual perception in recipients of electronic retinal prostheses.

POS-THU-134

EFFECTS OF SPATIAL FREQUENCY ON PERCEIVED CONTRAST FOLLOWING CONTRAST ADAPTATION IN HUMANS

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Perceived contrast is reduced after prolonged exposure to a textured pattern (contrast adaptation). The size of this effect is dependent on the relationship between the adapting contrast and the test contrast. It is generally accepted that the greatest reductions occur when the adapting contrast is much higher than the test contrast. **Purpose:** To examine the relationship between adaptation and test contrasts at a wide range of spatial frequencies (SFs). **Methods:** Five subjects (ages 21 to 43) viewed vertically oriented sinusoidal gratings with SFs of 0.18, 0.4, 1.8 or 3 cycles per degree (cpd). The temporal profile of the stimulus consisted of an initial adaptation phase (30s) immediately followed by a series of 40 tests (0.5s) interspersed with a top-up adaptation stimulus (4s). Following the test phase subjects reported which stimulus had "higher contrast" in a forced choice manner. In this way we generated an estimate of the perceived contrast using interleaved QUEST staircases. **Results:** We show that the effect of the adapt/test ratio on perceived contrast following contrast adaptation is highly SF dependent. At high spatial frequencies >1cpd perceived contrast was reduced for all adapting contrasts, which is consistent with other studies. However, at low spatial frequencies (<1cpd) the perceived contrast was actually above veridical perception when the adapting contrast was lower than the test contrast. **Conclusion:** This finding has not been previously reported and has important implications for models of contrast perception. The differential inputs of the magnocellular and parvocellular pathways and the contrast gain control of visual neurons are discussed as possible mechanisms producing these SF dependent contrast adaptation effects.

POS-WED-135

PRIMARY VISUAL CORTEX CONTAINS INFORMATION ABOUT COLOUR-FORM CONJUNCTIONS

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Purpose: The processing of colour and form is largely segregated within the visual brain. But there is also evidence to suggest that these features are intimately coupled and even coded in combination early in visual processing. Here we examined where in the visual cortex colour-form conjunctions are represented. **Methods**: Human subjects (n = 5) viewed visual displays containing coloured spiral patterns. The spiral patterns could be red or green, and oriented either clockwise or counter-clockwise, leading to four possible stimulus combinations. Two additional displays comprised two of the above single colour-form pairings that alternated at a frequency of 1Hz. We applied multivariate classifiers to voxel activation patterns obtained whilst subjects viewed such displays. **Results**: As well as confirming the representation of colour and form information across visual cortex, our analyses provided evidence for the explicit coding of conjunctions of form and colour as early as primary visual cortex. The voxels most informative about conjunctions were distinct from the voxels most informative about colour or form alone. **Conclusions**: The results of this study have implications for theories concerning the segregation and binding of colour and form information, as early conjunction coding might appear to render a late binding stage unnecessary.

POS-THU-136

DECODING HIGHER ORDER COLOUR SIGNALS IN HUMAN VISUAL CORTEX

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Purpose: We used fMRI to test for higher order cortical representations of colour capable of classifying stimuli that cannot be distinguished by the postulated red-green (L-M) and violet-yellow (S) subcortical opponent channels. **Methods:** Subjects (n = 5) viewed each of two patterns modulating in colour between orange-cyan or lime-magenta. Multivariate pattern classifiers restricted to each of several visual areas were trained to discriminate the two patterns. The classifiers were trained on signals from 9 trials and tested on a tenth; this procedure was repeated 10 times. **Results:** Classifiers performed significantly better than chance - impossible on the basis of signals from the opponent channels alone - as early as V1. **Conclusion:** The success of the classifiers implies: (1) subcortical chromatic channels are recombined early in cortical processing to form novel representations of colour; (2) non-uniform spatial mapping of these representations of colour in striate and extrastriate cortical areas.

POS-WED-137

PICKING SIGNAL FROM NOISE: INTERACTIONS BETWEEN MOTION DIRECTIONS

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Purpose. The visual system can detect coherent motion in the midst of motion noise. This is accomplished with motion-sensitive channels, each of which is tuned to a limited range of motion directions. Our aim was to show how a single channel is affected by motions both within and outside its tuning range. **Methods.** We used a psychophysical reverse-correlation procedure. An array of dots moved coherently with a new direction every 14 ms. There were 20 directions distributed evenly across the full 360° range, and each movement was randomly chosen from this set of directions. Human subjects (n = 4) were required to press a key whenever they saw movement in a target direction (vertically upwards). The results were analysed by finding, for each key-press, two motion directions: the first preceded the key-press by the reaction time, and the second preceded the first by 14 or 28 ms. **Results.** There were three main findings. First, the subject was significantly more likely to press the key when the vector average of the two motions was in the target direction. This indicates that two motions bracketing the target direction can sum, enhancing the output of the target detector. Second, a key-press was less likely when a target motion was preceded by a motion in the opposite direction. We interpret this as suppression of the detector's response by motion in its null direction. Third, both of these effects were short-lived in that they were only seen for inter-stimulus intervals of 28 ms or less. **Conclusion.** The results support the idea that a motion detector can sum sub-optimal inputs, is suppressed by motion in the null direction, and has a highly transient response.

POS-THU-138

UV-SENSITIVE OPTOMOTOR NEURONS IN HONEYBEES

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If an insect is placed in a rotating drum lined internally with textured patterns it reflexively turns its body to follow the patterns. In so doing the animal maintains its orientation relative to the visual environment. This optomotor reflex has been observed in all insects and the wide-field direction-selective optomotor neurons that drive the reflex have been located. In the mid-1970s Kaiser established that the spectral sensitivity of optomotor responses in bees almost exactly matched that of the green photoreceptors, suggesting an exclusive drive from green photoreceptors. **Results:** In contrast to this dogma, we have identified optomotor neurons in bees that respond more strongly to UV stimulation than to green stimulation. The green photoreceptors in bees have spectral sensitivities that stretch into the UV region of the spectrum but the UV-responses are very weak. Given the very strong UV-responses in our identified optomotor neurons, the results suggest a strong contribution from UV- photoreceptors. Our stimuli were restricted to dorsal eye regions while Kaiser's experiments stimulated the ventral eye. **Conclusion:** It appears that UV channels are important in optomotor responses in bees but that the sensitivity is selective for certain eye regions.

POS-WED-139

Cancelled

POS-THU-140

VISUAL PHYSIOLOGY OF CARCHARHINID SHARKS: MOTION PERCEPTION AND IRRADIANCE SENSITIVITY

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Purpose: The eyes of sharks in the main are well adapted for vision under dim light conditions and show an enhanced sensitivity to light. High light sensitivity requires both temporal and spatial summation which necessarily degrades the eye's ability to detect fast visual stimuli without blur. Many shark species are agile predators of mobile prey. The ability to detect and track moving objects is, therefore, an important visual task. Yet the temporal aspects of vision in sharks have received little attention. **Methods:** We examine the temporal response properties (indicated by the flicker fusion frequency~ FFF) and the irradiance sensitivity of photoreceptor cells in the tiger shark (*Galeocerdo cuvier;* n=3) and the sandbar shark (*Carcharhinus plumbeus;* n=30) using electroretinograms (ERG). **Results and conclusions:** Photoreceptor intensity-response curves conform to the Naka-Rushton model of photoreceptor function. Tiger shark photoreceptors (k50=-2.0±0.2) are maximally sensitive to a brighter range of light intensities than the sandbar shark (k50=-3.2±0.2) and implies the tiger shark occupies a more photopic light environment than the sandbar shark. FFF values for both species are shown to directly related to stimulus light intensity and contrast. The sandbar shark, however, has a faster visual system (maximum recorded FFF = 54 Hz) than the tiger shark (maximum recorded FFF = 38 Hz). This reflects a differential importance of motion perception between the two species and suggests the sandbar shark employs a visual strategy to maximise temporal resolution. Our study further characterises the role of vision in shark behaviour and highlights the possible impacts of anthropogenic environmental modifications on the sensory abilities of sharks.

POS-WED-141

RESPONSE OF SEA TURTLES HATCHLINGS TO LIGHTS OF DIFFERENT WAVELENGTH

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Purpose: Sea turtles' eyes are highly developed and are a key sense for these animals for a range of behaviours. For instance it has been shown in numerous studies that light stimuli are crucial to sea turtle hatchling's ability to find the sea after emerging from their nests on the beach. In this study the innate light-orienting behaviour of hatchling sea turtles was utilised to learn more about the animals' spectral sensitivity range. This study aimed specifically to establish visual thresholds for different wavelengths of light, revealing which wavelengths the animals are more sensitive to. **Methods:** Hatchlings of the loggerhead (*Caretta caretta*), green turtle (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*) sea turtle were tested in a Y-maze behavioural arena shortly after emerging from the nest. The animals had to choose between a dark and a dimly lit stimuli presented at the end of each arm of the maze. At the completion of the experiments the hatchlings were released onto the beach. **Results:** Hatchlings of all species tested here (n>500) responded to wavelengths between 365nm (UV light) and 700nm (red). All species were more sensitive to the shorter wavelength (representing colours such as UV and blue) than the longer wavelength (yellow and red) and species-specific differences of visual thresholds for different wavelength were found. Australian loggerhead sea turtles did not shown any avoidance behaviour to the colour yellow (600nm), unlike populations in Florida that were tested in a previous study (Witherington and Bjorndal, 1991). **Conclusion:** The research has implications for many of the man-made sources of lights that sea turtles encounter during their lifetime, such as beach lighting and luminous light sticks used in the longline fisheries. (Witherington and Bjorndal 1991. Copeia 4:1060-1069).

POS-THU-142

VISUAL ECOLOGY OF CEPHALOPODS

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Purpose The coleoid cephalopods (octopus, cuttlefish and squid) possess a remarkably advanced visual system which is sensitive to the e-vector of polarized light. Polarization sensitivity (PS) may allow them to communicate with conspecifics using polarizing patterns in their skin, navigate through the ocean and break the camouflage of transparent objects in the water. Their eyes bear similar structure to the vertebrate eye, and although mostly thought to be colourblind, coleoids possess a stunning repertoire of colourful body patterns. **Methods** Functional level PS was tested using vertically-striped barrels of cross-polarizing filter to test for the optomotor (OMR) and optokinetic (OKR) responses. Body pattern repertoires were also manipulated using substrates with different patterns and textures. Retinal mapping of photoreceptor density with whole mount techniques examined density and orientation of photoreceptors. **Results** Both OMR's and OKR's have been elicited in more than 20 animals from seven species. Coleoids possess areas of specialization in the retina corresponding to the shape of their pupil, whilst the arrangement of photoreceptors around the retina appears to follow a square, lattice-like array, allowing sensitivity to e-vectors entering the eye from any direction. **Conclusion** This study demonstrates functional level PS in several coleoid species for the first time. More species will now be tested using these methods. These results will be used to draw correlations between the features of the visual ecology of coleoid cephalopods with the characteristics of their habitats. This will in turn reveal whether phylogenetic of environmental pressures are driving the evolution of the visual system of these voracious predatory invertebrates.

POS-WED-143

ALIGNMENT OF SELF AND NON-SELF BALANCED BODIES

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Purpose: Studies in which subjects balanced a 'virtual body' to exclude vestibular input suggest that proprioceptive input from the legs is sufficient to stand and that vestibular inputs normally play no part in controlling body sway. However, there is no unique pattern of somatosensory input from the legs that signals the vertical alignment of the body. This study uses the virtual-body method to investigate whether proprioceptive input from the legs is sufficient. **Methods:** Balance was assessed under three conditions; normal standing, splinted standing to prevent rotation of joints above the ankle and balancing a virtual body (inverted pendulum), which simulated standing but excluded vestibular and graviceptive inputs. In each condition, subjects (n=12) were tested with and without additional weight attached to the body or pendulum. Sway, alignment angle and ankle torque were recorded. **Results:** When subjects stood normally, the position of the centre of mass of the body was approximately over the centre of the perimeter of the feet. This was maintained when a 30 kg weight was fastened around the pelvis (mean: 2.0±4.4mm). In contrast, adding 30kg to the virtual body with vestibular input unavailable caused the centre of mass to shift back towards the ankles (24.3±1.5mm).With only somatosensory input available, subjects balanced a lightweight virtual body over the front of the feet, their matched virtual body over the centre of the feet and a heavyweight virtual body over the heels. **Conclusions:** Vestibular and graviceptive sensory input, although not used to control sway during standing, has a unique role in aligning the body's centre of mass safely across the centre of the feet.

POS-THU-144

THRESHOLDS FOR DETECTING THE HEIGHT OF THE GROUND UNDERFOOT

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Purpose. To measure thresholds for detecting the height of the ground underfoot and to identify the sensory and motor processes involved. **Methods.** The *standing, stepping* and *walking* thresholds for detecting differences in the height of the ground between a test foot and a reference foot. Subjects (N=8) were tested while upright but not weight-bearing for both passive and active placement of the feet. Weight-bearing conditions included *standing* (standing freely and making height judgement), *stepping* (taking one step) and *walking* (five steps) onto a platform of variable height. To alter muscle sensory feedback, high-frequency (100Hz) vibration was applied transcutaneously to one tensor fascia lata muscle. **Results.** Height thresholds during non weight-bearing conditions were significantly greater than those during standing (means 8.8 and 5.8 mm respectively; $P<0.01$). The walking, but not the single step threshold, was significantly smaller than the standing detection threshold (means 3.9, 8.2 and 5.8 mm respectively; $P<0.01$). Vibration significantly offset the perceived ground level such that on the vibrated side, the ground felt lower (mean 5.5 mm). **Conclusions.** We are more sensitive to detecting the height of the ground when actively standing than when using non-balance proprioception, probably through use of additional graviceptive afferent and efferent signals. Walking further increases sensitivity, probably through use of temporal reafference processes. Proprioceptive signals from muscles are not only interpreted as changes in joint posture; a component of the signal is attributed to changes in the shape of the contact surface.

POS-WED-145

PROPRIOCEPTION WITH MUSCLE CONTRACTION AT THE HUMAN ANKLE

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Purpose. Movement detection with contraction is of interest because normal day-to-day movements inevitably involve contraction across one or joints. Studies on movement detection with muscle contraction thus far have produced inconsistent results. Movement detection has been shown to improve with active flexion of the forearm but worsen in the co-contracted forearm. These contradictory results may be attributed to the different contraction forces used in these studies or the differences in contraction and co-contraction. **Methods**. To explore these two possibilities, detection thresholds for movements imposed at a constant angular velocity of 1 deg/s were determined for the relaxed, contracted, and co-contracted ankle at four force levels of 13.5N, 27N, 54N and 108N. Sixteen healthy adults, aged 21-53 years, were studied. A Gaussian tracking curve was used to determine the lowest displacement amplitude at which movements were detected. **Results**. Movement detection was affected by different levels of muscle contraction and co-contraction. Compared to the passive thresholds, movement detection improved with low levels of contraction (10-20% MVC) and deteriorated with increasing contraction force. Similarly, weak co-contractions improved movement detection and worsened with stronger contractions. However, comparison of contraction and co-contraction thresholds shows that movement detection deteriorated more so with co-contraction at high force levels. **Conclusions**. These results suggest that afferent recruitment and therefore ensemble discharge from a population of muscle spindles occurs at relatively weak contractions, with a consequent reduction in movement detection at higher contraction levels. Differences in contraction and co-contraction thresholds point towards an involvement of spindle afferent inputs from both agonist and antagonistic muscles in movement detection.

POS-THU-146

PARALLEL MEASURES OF THE TONIC STRETCH REFLEX AND ITS MECHANICAL IMPACT

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Aim: To better understand the functional impact of the tonic stretch reflex (TSR) on joint mechanics, parallel measures were made of the reflex response to joint perturbation and resistive torque about the joint. These measures were then cross correlated over a broadband of stretch frequencies and a wide range of contraction levels. **Methods:** The metacarpal joint of the index finger was sinusoidally perturbed at sixteen frequencies (2.5 to 40 Hz) while the subjects (n=10) maintained a range of contraction levels (0-50% of MVC). Joint angle, surface electromyogram (EMG) from the first dorsal interosseoues muscle and angle-torque data were recorded. Cross correlation analysis was performed between angle/EMG and angle/torque, providing TSR and torque gain measures. Linear correlation was performed between TSR gain and torque gain across the population at every frequency. **Results:** TSR gain increased with stretch frequency and background contraction. Torque gain decreased from a maximum at 2.5 Hz until it reached a minima at 15-20 Hz and increased through the high frequency range. The joint thus had a pronounced resonance at this frequency. High correlation (r^2>0.45) between TSR gain and torque gain was found at all frequencies below ~20 Hz. **Conclusion:** The TSR contributed significantly to joint stiffness at frequencies below the resonant frequency and may therefore have a functional role in compensating for the nonlinear mechanical properties of the joint in this band.

POS-WED-147

PATHWAYS SUBSERVING THE TONIC VIBRATION REFLEX IN HUMANS

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Introduction: Vibration of the muscle tendon elicits a tonic contraction known as the tonic vibration reflex (TVR) both in man and the decerebrate cat. The TVR is believed to result from primary Ia spindle activity, which in the cat is highly sensitive to vibration and also group II spindle afferents. On the basis that the TVR may be lost after brain lesions, it has been attributed to a long loop pathway (1). The aim of the experiment was to explore the reflex pathway in the gastrocnemius and soleus muscles by controlled vibration of the Achilles tendon over a wide range of frequencies. Method: The surface EMG of the medial and lateral heads of gastrocnemius (GA) and soleus were recorded from eight healthy human subjects. A linear motor tracked digitally generated sinusoidal waveforms (Labview V7) so that the shaft delivered controlled vibrations to the Achilles tendon for 1 min at several frequencies (2.5-300 Hz). Results: Cross-correlation analyses were used to characterize the relationship between the vibration and rectified EMG signals in terms of their coherence, gain and phase difference. In all muscles, reflex gain and coherence were high in the frequency band below 100Hz. The plot of phase difference against frequency was linear below 100Hz in all subjects and its slope yielded a group mean latency estimate of 42ms. This latency approximately corresponds to the conventional GA tendon reflex. Conclusion: The TVR in the human calf muscles was characterized by a wide band response of maximal gain around 50Hz and of spinal delay in contrast to earlier reports. (1) Lee RG & Tatton WG (1975). Motor responses to sudden limb displacements in primates with specific CNS lesions and in human patients with motor system disorder. Can J Neuro Sci 2, 285-293.

POS-THU-148

INHIBITION OF THE STRETCH REFLEX COMPONENTS M1 AND M2 BY TENDON ELECTRICAL STIMULATION IN AN UPPER LIMB MUSCLE

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Introduction: Electrical stimulation of the Achilles tendon was recently shown to produce a powerful and prolonged (>250ms) presynaptic inhibition of the tendon reflex in both heads of gastrocnemius (GA) (1). The current study aimed to reproduce this finding in an upper limb muscle. Methods: A linear motor (Pae series amplifier, LDS, UK) delivered constant brief stretches to the active (10% MVC) abductor pollicis longus (APL) muscle by taping the thumb. The resulting torque (ICP sensor signal conditioner, UK) and APL surface EMG short and long latency reflex components (M1 and M2) were recorded and time series averaged (Labview, v7). The timecourse of conditioning effects resulting from electrical stimulation (20mA, 0.2ms) (Digitimer DS7) of the APL tendon on M1 and M2 was then investigated. Results: Mechanical perturbation of the thumb produced an M1 in APL of peak latency approximately 30ms and M2 of peak latency approximately 60ms. Tendon electrical stimulation strongly inhibited both M1 and M2 components over a period of approximately 80ms. Conclusion: No evidence of a prolonged presynaptic inhibition of the TR, as observed in the lower limb, was obtained. It seems likely that M1 and M2 were inhibited by a common spinal mechanism. (1) Khan SI & Burne JA (2008). Inhibitory mechanisms following electrical stimulation of tendon and cutaneous afferents in the lower limb. PhD Thesis, University of Sydney.

POS-WED-149

HISTORY-DEPENDENT CHANGES TO PASSIVE MUSCLE PROPERTIES IN RHEUMATOID ARTHRITIS

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Introduction: Subjective ratings of muscle stiffness in rheumatoid arthritis (RA) patients are increased after long periods of inactivity. It is possible that the thixotropic (or history dependent) properties of muscles may contribute to this type of stiffness. A study of the thixotropic properties of RA affected muscle is reported here. **Methodology:** Resistive torque and joint angle data were obtained (LabVIEW, v7) from RA patients (n = 13) and controls (n = 12) during sinusoidal displacement of the metacarpophalangeal joint in the abduction-adduction plane after rest intervals of 20, 40, 60 and 80 seconds, during which the joint was stationary and the muscles relaxed. The rest periods were preceded by high-amplitude perturbations (stirring movements) aimed at abolishing the history of muscle activity. By plotting the resistive torque against the joint displacement a hysteresis loop was obtained. The slope of the loop represented the elastic torque and the area of the loop, the viscous torque component. **Results:** The mean hysteresis area showed a linear increase with increasing rest interval in patients. The individual r^2 values averaged 0.87 for patients and 0.52 for controls. The mean area was larger in patients ($3.0 \pm 1.0 \times 10^{-4}$ Nm.deg) than controls ($6.0 \pm 2.0 \times 10^{-5}$ Nm.deg) ($F(4,20) = 3.8864$, $p<0.05$). The mean slope of the hysteresis loop also increased with the rest interval (mean $r^2 = 0.82$ in RA, and 0.54 in controls) and was significantly greater in patients ($F(4,20) = 94.787$, $p<0.05$). **Conclusion:** This study demonstrated augmentation of history-dependent changes to passive joint stiffness in RA patients, inferring that muscle properties contributed to subjective stiffness ratings. The result also suggests a possible interaction between the viscous and elastic components.

POS-THU-150

FIRING OF HUMAN INTERCOSTAL MOTONEURONES DURING QUIET AND VOLUNTARY BREATHING

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Purpose: Human respiratory muscles can be activated automatically by medullary centres, and voluntarily by the motor cortex. In quiet breathing, inspiratory intercostal muscles have a rostrocaudal gradient of activation, but the recruitment patterns in voluntary breaths are unknown. **Methods:** We compared single motor unit behaviour in the first, third, and fifth parasternal intercostal muscles in 5 subjects for quiet and matched voluntary inspirations. **Results:** Phasic inspiratory activity, relative to airflow, began after ~5% of inspiratory time (Ti) in the first interspace, but was delayed in the fifth space until ~32% of Ti, for quiet breaths ($p<0.05$). In voluntary breaths, activity commenced earlier, at the onset of Ti in the first interspace, and after ~25% Ti in the fifth space ($p=0.06$). During quiet breathing, the average peak firing frequency of motor units (n=244) was 13.2±0.9 Hz (mean±SEM) in the first interspace, but lower in the fifth space (9.4±1.0 Hz, $p<0.05$). For voluntary breaths (n=264 units), the peak discharge rate was 15.0±1.3 Hz in the first space, and 11.2±1.2 Hz in the fifth space ($p=0.07$). For 194 inspiratory units that were active in both tasks, the discharge frequency was ~20% higher in voluntary breaths than in quiet breaths. However, when frequency was adjusted for differences in tidal volume, airflow and the ratio of rib cage to abdominal movement, there was no difference in firing rate for quiet and voluntary breaths. Neural drive to the different parasternal intercostal muscles was correlated with their inspiratory mechanical advantage for both quiet and voluntary breaths ($r^2 \geq 0.99$). **Conclusion:** During voluntary breathing, there is a rostrocaudal gradient of motor unit activation across the parasternal intercostal muscles related to their mechanical advantage.

POS-WED-151

SUBTHRESHOLD CORTICAL STIMULATION INHIBITS HUMAN INSPIRATORY MUSCLES DURING VOLUNTARY BUT NOT INVOLUNTARY BREATHING

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The neural drive to respiratory motoneurones derives from at least two major pathways: bulbospinal output from respiratory centres in the medulla for automatic breathing, and corticospinal pathways. **Purpose:** The current study used transcranial magnetic stimulation (TMS) to inhibit corticospinal output (1) to determine whether the cortex drives respiratory muscles during voluntary breathing. **Methods:** In 7 subjects, electromyographic activity (EMG) was recorded via surface electrodes from the scalene muscles in the neck (obligatory inspiratory muscles). Low-intensity TMS (45-60% stimulator output) was delivered over the motor cortex during three conditions: (i) voluntary static inspiratory efforts against a closed airway, (ii) hypocapnic voluntary ventilation (end-tidal CO2, 2.5±0.1%), and (iii) hypercapnic involuntary ventilation (end-tidal CO2, 5.9±0.3%) induced by partial rebreathing. At least 50 stimuli were delivered at matched EMG levels in each condition. Stimulus intensity was chosen such that no short-latency facilitation, but only suppression, occurred in the rectified averaged EMG of 50 trials in static efforts. **Results:** Short-latency (16.7±0.5 ms) suppression of scalene EMG occurred during the voluntary tasks. Scalene EMG was reduced to 76±2% and 77±3% of ongoing EMG in voluntary breathing and static inspiratory efforts, respectively ($p<0.05$). During involuntary ventilation, EMG was not significantly suppressed (9±4%). This differed from the suppression during the two voluntary tasks ($p<0.05$). **Conclusion:** With differences in the chemical drive to breathe, TMS shows differences in the cortical contribution to breathing. Thus, during involuntary breathing, low-intensity TMS does not suppress the EMG activity. The study provides direct evidence that rapidly-conducting motor cortical output drives voluntary breathing. (1). Butler et al. (2007) JPhysiol 584, 651-659.

POS-THU-152

PLASTICITY IN THE HUMAN SPINAL CORD ALTERS VOLUNTARY MOTOR OUTPUT

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Purpose: Repeated delivery of pairs of timed pre- and post-synaptic action potentials induces lasting potentiation or depression at many synapses[1]. Delivery of pairs of stimuli designed to produce plastic changes at human corticospinal-motoneuronal synapses alters EMG responses to corticospinal tract stimulation[2]. We hypothesized that similar conditioning would alter normal physiological output. **Methods:** In 3 experiments, subjects (*n*=10) made brief (1-2 s) bilateral contractions of 10% maximal force with feedback of left arm force only. Contractions were performed before and after conditioning with 50 pairs of stimuli (0.1 Hz) designed to alter synapses in the corticospinal pathway to muscles of the right arm. In each stimulus pair, electrical brachial plexus stimulation evoked a maximal M-wave and transcranial magnetic stimulation (TMS) elicited a small motor evoked potential in biceps. On each day, different interstimulus intervals (ISI) were used, with TMS before peripheral stimulation (+3 and +22 ms) or vice versa (-13 ms). **Results:** Prior to conditioning, subjects made similar contractions with the two arms. As predicted from responses to corticospinal tract stimulation, when subjects made bilateral contractions at 28-44 mins after conditioning at -13 ms and +22 ms ISIs, force output from the conditioned right arm was decreased by 9±12% and 11±14% relative to the control arm (*P*<0.05). In bilateral contractions performed 8-24 mins after conditioning at the +3 ms ISI, right arm force was increased by 15±20% (*P*<0.05). **Conclusion:** Changes in corticospinal transmission which persist for at least 30 mins can be induced acutely by controlled synaptic activation. Alteration of voluntary motor output shows that these plastic changes are functionally relevant. (1) Dan & Poo (2004) Neuron 44:23-30 (2) Taylor & Martin (2008) Proceedings of ANS 18:p49.

POS-WED-153

VOLUNTARY ACTIVATION OF THE DIFFERENT COMPARTMENTS OF THE FLEXOR DIGITORUM PROFUNDUS

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Voluntary activation (VA), the level of neural drive to muscle during exercise, can be estimated by using transcranial magnetic stimulation (TMS) to evoke increments in force during voluntary contractions (Todd et al. 2003). The neural drive to muscles increases with increasing contraction force, causing a decrease in the superimposed twitch (SIT) that is minimal during a maximal voluntary contraction (MVC). VA varies between muscles, but it is not known whether it varies within a muscle with different compartments. Flexor digitorum profundus (FDP) is such a compartmental multi-tendoned muscle, flexing the fingertips of the four fingers. **Purpose:** The present experiment determined whether VA in the four compartments of FDP could be measured separately and whether VA differed between the fingers. **Methods:** Each finger was confined in a set-up which allowed only FDP to produce force at the fingertip. During isometric voluntary contractions of 10-100%MVC, TMS over the motor cortex evoked SITs. **Results:** Inverse linear relationships between voluntary force and SITs were seen for contractions between 30% and 100%MVC (r>0.9). Resting twitches were estimated by extrapolation of the regressions and used to calculate VA. VA of FDP for all fingers was ~92%, with no differences between fingers (repeated-measures ANOVA: p=0.23). Estimated resting twitches were biggest in the middle finger and the smallest in the index (p=0.002); the middle finger also had the highest MVC and little and index fingers the lowest (p=0.006). **Conclusion:** VA was successfully measured for the individual compartments of FDP and is comparable to VA of elbow flexors (~93%). Despite differences in MVCs and estimated resting twitch sizes, VA of the four fingers was the same. Todd, Taylor, Gandevia, 2003, J.Physiol 551:661-71.

POS-THU-154

THE EFFECT OF MUSCLE FATIGUE ON LONG-INTERVAL INHIBITION

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It is known that high-intensity *conditioning* transcranial magnetic stimulation (TMS) inhibits a second *test* stimulus delivered 100ms later. However, it is largely unknown how fatigue affects this inhibition. **Purpose:** To delineate cortical and spinal contributions to the inhibition in an actively contracting muscle during and after a sustained maximum voluntary contraction (MVC). **Methods:** Eight healthy subjects performed a 2min MVC of the elbow flexors. The motor cortex was activated by TMS delivered over the vertex. Single test and paired conditioning-test stimuli were delivered every 7-8s throughout the MVC and then during intermittent brief MVCs in the recovery period. On a separate day, subjects repeated the protocol but the TMS test pulse was replaced with electrical cervicomedullary stimulation between the mastoids. TMS motor evoked potentials (MEPs) and cervicomedullary evoked potentials (CMEPs) were recorded from the biceps brachii. Inhibition was calculated as the size of the conditioned test response divided by the unconditioned test response. **Results:** Unconditioned (single test) MEPs increased progressively with fatigue, whereas CMEPs increased initially but returned to baseline in the final 40s of the MVC. Conditioned (paired) MEPs and CMEPs decreased rapidly with fatigue and were virtually abolished in 30s. Inhibition increased with fatigue and mirrored the time course of the conditioned responses. In recovery, unconditioned responses required <30s but the conditioned responses required 90s to return to control levels. The MEP inhibition lasted 105s, whereas the CMEP inhibition was still below the control level at 285s. **Conclusion:** Long-interval inhibition increased with the progression of fatigue. Contrary to expectations, the effects occurred primarily at the motoneurones rather than the motor cortex.

POS-WED-155

FACILITATION OF THE HUMAN MOTONEURON POOLS IN THE TIBIALIS ANTERIOR MUSCLE AFTER MAXIMAL CONTRACTION

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Purpose: The corticospinal pathway is the major pathway controlling human voluntary movements. After strong voluntary contractions, the efficacy of corticospinal transmission to the elbow flexor muscles is reduced for ~90 s, and this reduces voluntary motor output (1). The current study investigated whether similar changes occur in a leg muscle, tibialis anterior (TA). Methods: In human subjects, electrical stimuli (100 µs pulse, 750 V) delivered through surface electrodes over T1-T2 and T3-T4 vertebrae activated axons in the corticospinal tract. EMG responses were recorded from right TA through surface electrodes. Stimuli were delivered before and up to 30 min after a 10-s maximal voluntary contraction (MVC) of ankle dorsiflexors. In the first study (n=10), stimuli were given with the muscle relaxed and, in the second study (n=8), during a weak contraction (5% MVC). Results: In the first study, following the 10-s MVC, there was an immediate large (variable) increase in size of the thoracic motor-evoked potentials (TMEPs) to 365±337 % of control values. By 1 min after the contraction, TMEPs decreased to 33±19% of control (p<0.001) and remained depressed for >10 min. In the second study, a less pronounced increase to 135±25% (p<0.05) and decrease to 68±27% (p<0.05) occurred over the same time course during weak contractions. Conclusion: The immediate facilitation and long-lasting depression in corticospinal transmission to TA after an MVC differs from changes seen in the elbow flexors. The occurrence of these changes during maintained motoneurone pool activation suggests that they originate at a premotoneuronal site. 1. Petersen et al. 2003 JNeurosci 23, 7974-7980.

POS-THU-156

MICROARRAY ANALYSIS OF THE INJURED SPINAL CORD OF EPHA4 KNOCKOUT MICE

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Mice lacking the axon guidance molecule EphA4 show extensive axonal regeneration following spinal cord hemisection. Alterations in the level of astrocytic gliosis and the vascular response to injury have previously been identified in EphA4 knockout mice, however the exact mechanisms underlying the regeneration remain unclear. **Purpose:** To identify which genes and downstream signalling pathways orchestrate the axonal regeneration seen in the EphA4 knockout mice. **Methods:** EphA4 knockout and wild-type mice aged between 10 and 14 weeks were used in the study. Four gender-balanced groups (n=3 per group) were included: EphA4 knockout and wild-type mice with either lumbar spinal cord hemisection or sham injury (laminectomy only). At four days post-injury a 6mm piece of tissue around the injury epicentre or equivalent was removed and snap-frozen. The RNA from each spinal cord was individually hybridised to an Affymetrix Mouse All-Exon Array 1.0 chip. Microarray data was analysed with Partek Genomics Suite© to identify differentially expressed genes. Significantly changed genes were further analysed using GOminer© to identify over-represented gene ontology groups. **Results:** A two-way ANOVA identified 90 genes with a genotype/treatment interaction (p≤0.01)(differentially expressed in response to injury in EphA4 knockout compared to wild-type mice). An alternative analysis of the data using genotype as a factor in a one-way ANOVA of injured animals only identified 94 significantly changed genes (p≤0.01) between EphA4 knockout and wild-type mice. A number of genes involved in the inflammatory response and sphingosine-1-phosphate pathway were identified. Subsequent analysis using GOminer© identified gene ontology groups which were over-represented in gene lists from both analyses; these included the IkappaB kinase/NFkappaB cascade and regulation of apoptosis (p≤0.04). **Conclusions:** We have identified a number of genes and processes which potentially contribute to the neural regeneration observed in injured EphA4 knockout mice.

POS-WED-157

IN-VIVO TESTING OF A NOVEL MIMETIC PEPTIDE AGAINST CONNEXIN43 IN RAT SPINAL CORD CONTUSION INJURIES

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Introduction: Connexin43 is a gap junction protein associated with astrocytes and known to be up regulated after spinal cord injury resulting in lesion spread to healthy cells and the opening of hemichannels that further exacerbates the injury. A novel mimetic peptide designed to interfere with these processes has been shown to decrease tissue swelling, reduce astrocytosis and promote neuronal cell survival in a concentration and time dependent manner in spinal cord explants[1]. We are testing this peptide for the first time in vivo, using a spinal cord contusion injury model. **Methods:** 32 rats were subjected to a 10g, 12.5mm weight drop injury at the vertebral level T10. An intrathecal catheter attached to an Azlet osmotic pump was used to deliver vehicle or Connexin43 peptide (5μM, 20μM or 50μM) to the lesion site at a rate of 8μl/hr for 24 hours. Animals were assessed for behavioural changes and killed at 6 weeks post injury for histological analysis of spinal cord tissues. **Results:** There were marked improvements seen in hindlimb locomotor function at 1 week for the 5μM and 20μM treatments although the improvements did not persist for the 6 week trial. There were reductions in lesion size ($p < 0.05$) and GFAP intensity with the 5μM treatment at 6 weeks. **Conclusions:** These results indicate the potential for hemichannel modulation, using perfused mimetic peptides, to improve outcomes following spinal cord injury. Further studies aimed at optimising the benefits, including peptide pharmacodynamics and the time course for treatment, are being undertaken to optimise these outcomes. 1.O'Carroll, S et al (2008) Cell Communication & Adhesion,15:1,27—42.

POS-THU-158

NATURAL KILLER CELLS ARE INCREASED FOLLOWING STEM CELL TRANSPLANTS IN THE ATHYMIC RAT SPINAL CORD

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Human stem cells are being trialled as a therapy to promote cellular and functional improvement after spinal cord injury. While athymic rats models are used to prevent xenograft rejection they show varying success for transplant cell survival. **Aim:** To examine the natural killer (NK) cell and macrophage response to 4 types of human cell transplants in the athymic rat spinal cord. **Methods:** 16 female athymic rats were assigned into one of four groups, each receiving a different type of cell transplant: human embryonic stem (hESC), bone marrow stem (hBMSC), olfactory ensheathing (hOEC) or olfactory progenitor (hOSC) cells. 2 injections of 0.5ul (50-100,000 cells/μl) were made at T10 and T11. After 1 and 7 days, sections were analysed for cell survival, asialo (NK) and ED1 (macrophage) immunoreactivity. A ranked scoring system was used to assess cell numbers. **Results:** At day 1 large and obvious cell transplants were noted for all cell types. By day 7, hESC and hBMSC numbers were reduced (Mann-Whitney test, $p<0.05$) with no sign of change for hOEC and hOSC. NK cell numbers were higher for hESC and hBMSC at day 7, compared to hOEC and hOSC. Macrophage cell numbers increased similarly in all groups by day 7. **Conclusion:** These results suggest NK cells are involved in transplant cell death. Further investigation into the mechanisms activating innate immune responses to human stem cells, hESCs and hBMSCs in particular, is warranted if the athymic rat model is to be used to trial therapies for human cell transplants.

POS-WED-159

IDENTIFYING GENES RELATED TO MOVEMENT DISORDERS IN MICE

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Purpose In collaboration with the Australian Phenomics Facility (APF), this project aims to identify genetic mutations that cause ataxia and other movement disorders in mice. APF run a large-scale ENU mutagenesis facility and provide us with access to a large number of mice carrying random point mutations in their DNA. Identifying genes causing movement disorders will improve our understanding of the mechanisms involved and potentially lead to new drug targets for human conditions such as motor neuron disease and ataxia. **Methods** Mice with movement disorders were selected by direct observation of behaviour. APF performed all genetic mapping. RNA was extracted from whole mouse brains using Trizol and reverse transcribed to produce cDNA. The chromosomal region was searched for candidate genes, and primers designed to amplify cDNA. cDNA from the mice was PCR amplified and screened for mutations by direct sequencing. **Results** Six mouse strains with observable movement disorders were selected for further study and the genes mapped to particular chromosomes. Brain tissue from these mice was used for RNA isolation and mutation screening. To date, the gene mutations have been identified in two of the six mouse strains. The genes are *Kcnn2* and *Clcn1*. **Conclusion** Screening ENU mutagenised mice provides a rapid means of identifying novel mouse models of human disease. We have identified two ion channel genes that cause movement disorders in mice. These two new mouse strains are an important resource for understanding the normal function of the *Kcnn2* and *Clcn1* genes, and how mutations in these genes lead to movement disorders.

POS-THU-160

SK2 MUTATIONS CAUSE A MOVEMENT DISORDER IN ENU-MUTAGENISED MICE

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Purpose: We aimed to characterise new mouse models for neurodegenerative disorders, such as motor neuron disease, by screening ENU mutagenised mice. Mapping genes in strains with movement disorders will provide new candidate genes for screening in human neurodegenerative disorders. **Methods:** An ENU mutagenesis strategy generated several strains of mice that exhibited movement phenotypes that may reflect a neurodegenerative disease. We initially focused on one strain, ENU6:28, that displayed non-progressive generalised tremors, mild hypermetria and jerky movements. **Results:** The ENU6:28 mutation was mapped to an 8 Mb region on chromosome 18. Candidate gene re-sequencing revealed a mutation in the *Kcnn2* gene that encodes the calcium-activated potassium channel, SK2. Analysis of brain cDNA from ENU6:28 mice revealed that the mutation caused skipping of exon 7, leading to deletion of the vital calmodulin binding domain (CaMBD) of the SK2 channel. **Conclusions:** SK2 is primarily expressed in somatic and dendritic structures of the neocortex, hippocampus and cerebellum and mediates the afterhyperpolarisation current that occurs following an action potential, regulating firing frequency of neurons. Loss of the CaMBD in the ENU6:28 strain is likely to represent a functional knockout model for the SK2 channel. Disruption of SK2 function in cerebellar Purkinje cells may explain the movement disorder observed in the ENU6:28 mice. Identifying genes that cause movement disorders in mice will improve our understanding of the mechanisms involved and potentially lead to new drug targets for human conditions such as motor neuron disease and ataxia.

POS-WED-161

VOLTAGE-GATED SODIUM CHANNEL GENE EXPRESSION IN A TRANSGENIC MOUSE MODEL OF MOTOR NEURONE DISEASE

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Purpose: Transgenic mice overexpressing a G93A mutation in the human superoxide dismutase 1 gene (hSOD1^{G93A} mice) show motor neurone hyper-excitability and increased persistent Na^+ current (PC_{Na}) from birth. We investigated levels of Na^+ channel gene expression in motor cortex, brainstem and lumbar spinal cord of wild type (WT) and hSOD1^{G93A} mice. **Methods:** WT and hSOD1^{G93A} mice aged P0, P7, P16, P28 and P71 (n>= 2-7 for each age and genotype) were anaethetised with sodium pentobarbitone (100 mg/kg i.p.) and tissue samples from motor cortex, brainstem (including the hypoglossal motor nucleus) and lumbar spinal cord were dissected out in ice cold ACSF. Real time RT-PCR using commercial Taqman probes directed against mouse $Na_V1.1$, 1.2, 1.3 and 1.6 genes and the housekeeping gene HPRT1 was done in triplicate using cDNA generated from tissue samples. **Results:** In WT mice, $Na_V1.1$ expression increased with age in cortex, brainstem and spinal cord, while $Na_V1.3$ expression was relatively low at birth and decreased further with age in these areas. $Na_V1.2$ gene expression was high at birth relative to other Na^+ genes in all areas, and declined moderately with age to lower levels. $Na_V1.6$ expression was moderate at birth and increased with age in cortex and brainstem. In hSOD1^{G93A} mice, $Na_V1.1$, 1.2, 1.3 and 1.6 expression was higher at birth and P7. $Na_V1.1$, 1.2 and 1.3 expression remained stable or declined at older ages, while $Na_V1.6$ expression remained elevated. **Conclusion:** hSOD1^{G93A} mice have altered expression patterns of Na^+ genes in brain areas involved in adult neurone loss in motor neurone disease, consistent with neuronal hyper-excitability due to increased expression of PC_{Na}.

POS-THU-162

MOTOR NEURON SPECIFIC, NON-VIRAL GENE DELIVERY TARGETED THROUGH THE P75NTR

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Purpose: Receptor specific, non-viral gene delivery vehicles provide a way to deliver therapeutic agents for neurological conditions such as motor neuron disease. We have established a highly effective non-viral gene delivery technique selectively targeting p75^{NTR} expressing motor neurons using NSC-34 SOD1^{G93A} cells, and primary embryonic motor neurons from the SOD1^{G93A} transgenic mouse. **Methods:** Monoclonal antibody to the common neurotrophin receptor p75 (MLR2) was conjugated to polyethylenimine (MLR2-PEI) and complexed with an eGFP expression plasmid to form the immunoconjugate, MLR2-PEI-pGFP. Target specificity, binding, uptake, and transfection efficiency were assessed using fluorescence microscopy in both the NSC-34 & NSC-34 SOD1^{G93A} cells, and in a control fibroblast cell line lacking the p75 receptor. Immunoconjugates were subsequently tested for their ability to transfect primary motor neurons from E12-13 wild type and SOD1^{G93A} transgenic mice. **Results:** MLR2-PEI-pGFP bound specifically to cells expressing the p75 receptor (n=3). The specificity was confirmed by the absence of delivery to cells lacking the p75 receptor and by competition studies with free ligand (MLR2) in NSC-34 cultures (n=3). In mixed cultures containing primary motor neurons and glia, only motor neurons were transfected (n=3). Finally, preliminary data indicates MLR2-PEI-eGFP was delivered to p75-expressing neurons in-vivo following intraperitoneal injections to adult mice (n=1). **Conclusion:** This study provides a powerful new approach to selectively manipulate motor neuron function *in vitro* and *in vivo*, opening up new therapeutic avenues in the treatment of diseases. Future work will include using this agent to deliver potential therapeutic agents to mice with motor neuron disease.

POS-WED-163

INVESTIGATING THE FUNCTIONAL CONSEQUENCES OF GENE MUTATIONS IN ALS

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ALS (amyotrophic lateral sclerosis) is an adult-onset neurodegenerative disorder that causes degeneration of both upper and lower motor neurons. The principal pathology of ALS is the presence of ubiquitin positive protein aggregates in the cell body of the motor neurons. TDP-43, principally a nuclear protein (encoded by TARDBP gene) protein is a major component of the UBIs (Ubiquinated inclusions) in ALS. TDP-43 pathology is present in all ALS (familial and sporadic) cases except SOD1-positive cases. Several TARDBP mutations have recently been reported in both familial and sporadic cases. **Purpose**: To screen for additional mutations in TARDBP gene among an extended familial ALS cohort (n=32). Another aim is to establish neuronal cell models expressing mutant TDP-43 and to investigate the functional consequences of these mutations in patient cells (lymphoblasts, fibroblasts and post-mortem tissues). **Methods**: PCR and sequencing techniques were used to screen the familial ALS cohort. Transfection, cell stressing methods, Immunohistochemistry, Immunofluorescence and Western blotting approaches are being employed to establish neuronal cell models and to the study of patient cells. **Results**: No new TARDBP gene mutations were found in the extended ALS cohort. The functional consequences of recently published TARDBP mutations (M337V, Q331K, G294A and G294V) are therefore being analysed. Our preliminary immunohistochemistry results show that there is an abnormal redistribution of TDP-43 from nucleus to the cytoplasm in Q331K, M337V and G294A mutations when different cellular stresses are induced. TDP-43 was shown to form aggregates in Q331K, G294A and G294V mutations. **Conclusion**: Abnormal redistribution and formation of aggregates suggests that normal function of the TDP-43 was disrupted. Work is underway to reproduce these results with immunofluorescence. Hyperphosphorylation and N-terminal cleavage of TDP-43 are currently being analysed with Western Blots. The effects of induced cellular stresses on patient lymphoblasts are also in progress.

POS-THU-164

GABA-DEFICIENT MICE PRODUCE REGIONAL CHANGES IN MOTONEURON SURVIVAL AND ACTIVITY DURING DEVELOPMENT

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Purpose: CNS GABAergic and glycinergic synaptic activity switches from excitation to inhibition during developmental motoneuron death. Our previous work on mice lacking glycinergic transmission, suggested that altered levels of motoneuron activity may help regulate motoneuron numbers during development (Banks et al., 2005). To investigate whether GABAergic transmission plays a similar role in neuromotor development, we quantified motoneuron number and activity in mice lacking GABA (GAD67-deficient mice). **Methods:** GAD67-deficient and wild-type embryos at the beginning (E13), middle (E15), and end (E18) stages of developmental motoneuron death were processed for motoneuron counts (see Banks et al., 2005; *n*=6/age). Motor pool activity was recorded using a brainstem-spinal cord preparation (Banks et al., 2005). We recorded C4-C7 and hypoglossal (XIIn) ventral root nerve activity via suction electrodes (*n*=7-10). **Results:** At E18, GABA-deficient mice showed a significant decrease in XIIn motoneuron numbers (1753 ± 40) compared to wild-type (2054 ± 100; *n*=6, *P*=0.013 *t*-test). This decrease in mutant motoneuron number was correlated with increased mean nerve burst frequency, burst amplitude and activity (charge/min) in XIIn nerves. By contrast, brachial motoneuron numbers in GABA-deficient mice were increased (3316 ± 97) compared to wild-type (2506 ± 102, *n*=6, *P*=0.0002, t-test). This increase in mutant motoneuron number was correlated with a decreased mean nerve burst frequency, burst amplitude and activity in C4-C7 nerves. **Conclusion:** Our present results suggest that during development, GABA-ergic transmission can regulate motoneuron numbers in a regional manner, similar to mice lacking glycinergic transmission (Banks et al., 2005).

POS-WED-165

LENGTH TENSION CURVE OF HUMAN SINGLE MOTOR UNITS

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Purpose: The amount of force a muscle produces is influenced by many factors including its length. The length tension relationship has been established for whole muscles and in animal studies at the level of the single sarcomere, muscle fibre and motor unit. This study investigated whether human single motor units demonstrate a length tension curve. **Methods:** Nineteen single motor units in either flexor digitorum superficialis or flexor pollicis longus were selectively studied using intraneural motor axon microstimulation. Peak isometric force was measured in response to single twitch (average of 5 pulses at 1Hz) and tetanic stimuli (mean force over 250 ms during 1s train at 50Hz) beginning in a neutral position with the hand and arm aligned longitudinally, then with either ~20° flexion or extension in a randomised order. **Results:** All motor units demonstrated a length tension curve in response to twitch stimulation, as did all but 3 for tetanic stimulation. Responses fell into one of three patterns with optimal length occurring in the neutral position (6 units for twitch stimulation, 2 for tetanic), in the flexion position (6 and 4, respectively) or in the extension position (4, 4). Significant differences between positions were found for all twitch curves ($p \leq 0.027$), and when optimal length occurred in the extension and flexion positions for tetanic stimulation ($p \leq 0.05$) but not in the neutral position. Only one motor unit showed a different pattern between twitch and tetanic stimulation. **Conclusions:** Single human motor units operate along a length tension curve. These results suggest that reported differences in the literature for peak forces of single motor units studied with intraneural microstimulation arose due to different muscle lengths associated with limb posture.

POS-THU-166

DIFFERENT TIME COURSES OF GABAB RECEPTOR MEDIATED POST- VS. PRESYNAPTIC INHIBITION IN HUMAN MOTOR CORTEX

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INTRODUCTION: Suprathreshold TMS can evoke long-interval cortical inhibition (LICI) mediated by GABAb receptor activation. We have recently shown that this is followed by a period of long-interval cortical facilitation (LICF). This may correspond to a period of reduced inhibition via ongoing GABAb auto-receptor activation. Here we have used triple-pulse TMS to measure short-interval cortical inhibition (SICI) during and after LICI. We hypothesized that SICI will be reduced by GABAb auto-receptor activation and that this will outlast GABAb receptor activation (LICI) and correspond to a period of cortical facilitation (LICF). **METHODS:** In 7 healthy subjects (19-38yrs), MEP amplitude was measured following paired- or triple-pulse TMS (priming, conditioning and test stimuli; PS/CS/TS) delivered at PS-TS intervals from 100-300ms. PS intensity gave ~1mV MEP. TS intensity was set to PS, as well as adjusted at each PS-TS interval to give ~1mV MEP. CS were delivered 2ms before TS so as to give 50% SICI without PS. **RESULTS:** There was significant LICI for up to 190ms after PS ($p<0.05$), followed by a period of LICF lasting ~50ms and maximal 200-220ms after PS ($p<0.05$). SICI was significantly disinhibited from 100-220ms after PS ($p<0.05$). **CONCLUSION:** Taking LICI as a measure of post-synaptic GABAb receptor-mediated inhibition, and the reduction in SICI as a measure of GABAb autoreceptor-mediated disinhibition, the results indicate auto-receptor activation outlasts receptor activation and corresponds to a period of cortical facilitation. The dynamic interaction between SICI and LICI suggests a role for auto-receptor activation in modulating cortical inhibition.

POS-WED-167

MOTOR CORTEX PLASTICITY INDUCED BY PAIRED ASSOCIATIVE STIMULATION IS ENHANCED IN PHYSICALLY ACTIVE INDIVIDUALS

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Purpose: Recent evidence indicates that regular physical activity enhances brain plasticity (i.e. the ability to reorganise neural connections) and improves learning and memory. However, the effect of physical activity on human primary motor cortex (M1), which plays a vital role in voluntary movement and motor learning, is unknown. The purpose of this study was to examine neuroplasticity in M1 of highly active and sedentary individuals. **Methods:** Electromyographic recordings were obtained from the left abductor pollicis brevis (APB) muscle of 14 active and 14 sedentary subjects (aged 18-38 yrs). The physically active group performed >150 min/day moderate-to-vigorous aerobic activity at least 5 days/week, whereas the sedentary group performed <20 min/day of physical activity for no more than 3 days/week. Transcranial magnetic stimulation (TMS) of the right hemisphere was used to assess neurophysiological parameters of plasticity, such as changes in APB motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI). Neuroplastic changes were induced using paired-associative stimulation (PAS), which consisted of 90 paired stimuli (0.05 Hz for 30 mins) of median nerve electrical stimulation at the wrist followed 25 ms later by TMS. **Results:** Despite similar MEP amplitudes between groups before PAS (~1 mV), there was a significant increase in MEP amplitude in the physically active subjects after PAS (54% increase compared with before, $P<0.01$), but no significant facilitation in the sedentary subjects (6% increase). SICI was not influenced by PAS for both groups. **Conclusion:** These findings suggest that regular physical activity increases neuroplasticity in human M1, which may be beneficial for learning new motor skills and in recovery of function following brain injury.

POS-THU-168

LANGUAGE TASKS INCREASE HAND MOTOR CORTEX EXCITABILITY WITHOUT ALTERING INTRACORTICAL INHIBITION

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Purpose: The hand area of human primary motor cortex (M1) is activated during speech production and perception[1], however the underlying mechanisms are not clear. We investigated whether GABAergic intracortical inhibition is modulated in hand M1 during language-related tasks. **Methods:** M1 was investigated with focal transcranial magnetic stimulation (TMS) in 21 (9M, 12F; age 18-50 years) right-handed (LQ > 0.85) subjects. Surface EMG was recorded from left and right first dorsal interosseous (FDI) muscles. Single- and paired-pulse TMS (3-ms interval) were used to assess motor evoked potential (MEP) amplitude and short-interval intracortical inhibition (SICI) for FDI of each hand at rest during six tasks (sitting silently, listening to white noise, reading silently, listening to speech, reading aloud, and reading aloud in unison with another speaker- "chorus speech"). **Results:** MEP amplitude was larger during reading aloud and chorus speech compared to reading silently, listening to white noise and sitting silently ($P < 0.05$). MEP amplitude was larger while listening to speech *vs*. reading silently ($P < 0.05$). There were no significant differences in this pattern with TMS of left or right hemisphere ($F_{1,100} = 0.34$, $P = 0.57$). There was no significant modulation of SICI during the six tasks ($F_{5,100} = 1.45$, $P = 0.21$). **Conclusion:** Corticomotor excitability of hand M1 is facilitated bilaterally during speech and listening tasks, but SICI is unchanged in hand M1 bilaterally during all language-related tasks examined. We conclude that modulation of intracortical inhibition does not contribute to the changes in hand M1 excitability during speech production and perception. 1. Floel A. *et al.* (2003) Eur J Neurosci. 18:704-8.

POS-WED-169

GROUP III METABOTROPIC GLUTAMATE RECEPTOR AGONISTS DECREASE GLUTAMATE RELEASE IN THE RAT SUBSTANTIA NIGRA AND PROVIDE FUNCTIONAL NEUROPROTECTION AGAINST 6-OHDA-LESIONS

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Purpose: Increased glutamate release in the substantia nigra (SN) contributes to motor symptoms and neurodegeneration in Parkinson's disease (PD). We investigated whether activation of nigral group III metabotropic glutamate receptors (mGlu4, 7 and 8) inhibited glutamate release in the SN and protected against 6-OHDA-lesions. **Methods:** Male rats had microdialysis probes implanted into the SN. KCl was administered via the probe evoking neuronal glutamate release. Following a 100-minute washout KCl was administered alongside L-SOP (group III mGlu agonist). Separate rats were cannulated above the SN. One week later rats received 6-OHDA intranigrally. L-AP4 (group III agonist), PHCCC (mGlu4 postive modulator) or AMN082 (mGlu7 agonist) were also given intranigrally for 7 days. On day 6 the number of adjusted steps made by ipsilateral and contralateral paws were counted. On day 7 animals were perfused and brains processed for striatal tyrosine hydroxylase-immunoreactivity (TH-ir). **Results:** L-SOP (300µM, N=4) caused a significant reduction (-48%) in glutamate release. In 6-OHDA rats, L-AP4 (10nmol, N=7) caused a significant increase in contralateral paw use, with the number of steps being 74 % of pre-lesion levels, compared to 13% in vehicle-treated animals. Furthermore, rats treated with L-AP4 showed significantly greater (+24 %) levels of striatal TH-ir compared to vehicle treatment. PHCCC and AMN082 failed to show any significant changes in paw use or TH-ir. **Conclusion:** These data confirm group III mGlu receptors decrease glutamate release in the SN. They also indicate activation of mGlu4, 7 and 8 together, but not mGlu4 or 7 alone, can protect against nigral degeneration with accompanying improvements in motor function. Hence group III mGlu receptors may provide neuroprotection in PD.

POS-THU-170

IS WNT1 EXPRESSION A CHARACTERISTIC OF ALL MOUSE HINDBRAIN PRECEREBELLAR NUCLEI?

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Purpose: Almost of the precerebellar nuclei are located in the hindbrain; their neurons are born in the rhombic lip from which they migrate to their definitive position. *Wnt1* is strongly expressed in the rhombic lip and has been showed to be expressed in the major precerebellar nuclei. **Methods**: To determine whether *Wnt1* expression characterises all hindbrain precerebellar nuclei, we compared the detailed pattern of *Wnt1* expression in Wnt1-Cre; ROSA26R reporter P0 mice, with the pattern of cell groups that were labeled following a series of HRP injections into the cerebellum (HRP traced mice, n=24). **Results:** We found that the following precerebellar nuclei (as defined by HRP uptake) showed *Wnt1* expression: the pontine nuclei, rhombencephalic reticular formation (reticulotegmental nucleus of the pons, lateral reticular nucleus, linear nucleus, and paramedian reticular nucleus), solitary nucleus, perihypoglossal nuclei, cuneate nucleus, external cuneate nucleus, spinal trigeminal nuclei, vestibular nuclei, nucleus X, inferior olivary nucleus, and the parvicellular part of the motor trigeminal nucleus. **Conclusion**: *Wnt1* is strongly expressed in cerebellar granule cells as well as Purkinje cells, and it seems likely that it plays a role as a guidance marker in establishing the circuitry that links the hindbrain with different layers of the cerebellum.

POS-WED-171

PROJECTIONS FROM THE CUNEIFORM COMPLEX TO THE SPINAL CORD IN THE MOUSE

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To identify the cells origin of the cuneiform complex projecting to the spinal cord in the mouse. Microinjections of retrograde tracer HRP was made in the cervical spinal cord between C1 and C2 in 25 C57/BL6 mice, followed by TMB staining. We have identified over 30 significant cell groups that project to the spinal cord in the mouse. We have confirmed that the pattern of major projections is typically mammalian, and very similar to that found in the rat. However, a novel finding was the prominent and continuous line of cells stretching from the cuneifom complex through the mesencephalic reticular formation to the caudal part of prosomere 1 in sagittal sections. The cuneiform complex consists of four distinct regions-the precuneiform nucleus (PrCnF), the dorsal cuneiform nucleus (CnFD), the intermediate cuneiform nucleus (CnFI) and the ventral cuneiform nucleus (CnFV). These can be distinguished on the basis of AChE staining. Only the PrCnF contained the labeled cells. The other three subnuclei of the CnF complex did not appear to project to the spinal cord. The cuneiform region has been identified as the major locomotor pattern generator in mammals ('the mesencephalic locomotor region'), our data suggest that the PrCnF is the most important in locomotor action, the other CnF subnuclei may serve other functions like autonomic control.

POS-THU-172

KNOCKDOWN OF ANGIOTENSIN PRECURSOR PROTEIN IN VITRO

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The classic Renin-Angiotensin System (RAS) acts systemically to potently regulate blood pressure and fluid homeostasis by the RAS product angiotensin II interacting with its receptor. This system is also known to exist independently in the brain, although current methods are inadequate to resolve the relative importance and anatomy of the tissue RAS in vivo without confounding by systemic RAS. **PURPOSE:** A novel approach to investigate the brain RAS is to knockdown endogenous gene expression of the only known angiotensin precursor, angiotensinogen (Ao), in specific cells using RNA interference technology. This technology uses short-hairpin RNA, complementary to a region of the gene of interest, which binds to the cell mRNA inducing degradation by endogenous DICER enzyme. **METHODS:** Three microRNA (mir) sequences were designed to complement distinct regions distributed along the rat Ao gene. Each mir sequence was ligated behind the ubiquitous cytomegalovirus (CMV) promoter into a plasmid vector. These plasmids were transfected into cultured HEK293 cells transiently expressing rat Ao. Cells were also transfected with a plasmid containing a mir with no sequence homology to any known gene sequence as a negative control. After 48 h cell proteins were extracted and analysed using SDS-PAGE followed by Western blot, probing for Ao and β-actin. **RESULTS:** Ao protein expression was reduced by two of three Ao-mir plasmids whilst no knockdown was observed in the negative control. **CONCLUSION:** RNA interference can be used to knockdown Ao protein *in vitro*. When combined with cell-specific promoters and viral vector delivery, this method provides an unprecedented ability to localise protein knockdown in specific cell types in discrete locations, of particular importance when examining specific brain nuclei.

POS-WED-173

DOES AUGMENTED RESPIRATORY-SYMPATHETIC COUPLING IN THE RAT CONTRIBUTE TO HYPERTENSION?

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Purpose. We have shown that respiratory coupling of sympathetic nerve activity (SNA) is increased in spontaneously hypertensive rats (SHR) even in early postnatal (pre-hypertensive) ages compared to normotensive Wistar-Kyoto rats (WKY). This is reflected by the larger Traube-Herring (TH) waves in SHR. We hypothesised that enhanced respiratory-sympathetic coupling in SH rats is not related to baroreflex feedback resulting from the TH waves but is a causal factor in the development of hypertension. **Methods.** PP, phrenic nerve activity (PNA) and thoracic (T8) SNA were recorded simultaneously in the working heart brainstem preparation of 5-week-old male SHR and WKY. A short period of apnoea was induced using a hypocapnic stimulus (2% CO2) followed by return to normocapnia (5% CO2) in WKY and SHRs (n=6 each). **Results.** Barodenervation in SHRs (n= 5; vagal and glossopharyngeal nerve section) did not alter respiratory-sympathetic coupling. Baseline PP and TH waves were greater in SHR (82.7±5.1 vs 65.2±1.2mmHg and 4.6±1.8 vs 2.3±0.8mmHg, respectively). Hypocapnia induced apnoea caused PP to fall by 7.3±3.6mmHg in SHR and 4.6±1.5mmHg in WKY. TH waves and respiratory related bursts in SNA were lost with apnoea. With the return of PNA to eupnoea, PP increased significantly more in SHRs than WKY (14.8±4.4 vs 4.5±1.7mmHg, $p<0.05$) but this only occurred after the re-emergence of respiratory related SNA bursting and TH waves. **Conclusions.** Enhanced respiratory SNA coupling in SHRs is reflected by greater arterial tone as revealed by elevated PP and TH waves compared to WKY rats. Thus increased respiratory-related bursts of SNA may, over time, be a causal factor in the development and maintenance of hypertension.

POS-THU-174

SPONTANEOUSLY HYPERTENSIVE RATS SHOW INCREASED EXPIRATORY ABDOMINAL MOTOR NERVE ACTIVITY WHICH CORRELATES WITH INCREASED SYMPATHETIC NERVE ACTIVITY

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Purpose. Rats submitted to chronic intermittent hypoxia (CIH) develop hypertension. Recently, we showed that CIH leads to an altered pattern of central respiratory-sympathetic nerve activity (SNA) coupling that correlates with enhanced late expiratory discharge recorded in the abdominal nerve[1]. Our aim was to assess whether such alterations in respiratory-sympathetic coupling were also found in the spontaneously hypertensive rat (SHR). **Methods.** Simultaneous recordings of phrenic, thoracic sympathetic, abdominal and cervical vagus nerves were made using the working heart brainstem preparation of 5-week-old SHR and Wistar Kyoto rats (WKY). **Results.** SHRs showed significantly higher levels of abdominal nerve activity overall compared to WKY (n=4 each; $p<0.01$) with an additional burst in late expiration and a smaller burst of post-inspiratory activity. The late expiratory abdominal discharge in SHR was coupled with an additional SNA burst in this phase of the respiratory cycle that was not evident in WKY rats. During increased respiratory drive with hypercapnia (10% CO2), expiratory abdominal activity was revealed in the WKY rat (and couple to SNA) and elevated in the SHR. Inspiratory related cervical vagus nerve activity was not significantly elevated in SHR but the proportion of activity during inspiration was significantly greater in SHR compared to WKY with a faster rise time to peak activity ($p<0.05$). **Conclusions.** As in CIH induced hypertension[1], we have found there is recruitment of late expiratory abdominal activity (active expiration) that provides additional respiratory modulation to sympathetic activity in the SHR which may contribute to the higher vascular resistance in this animal model. 1 Zoccal et al., 2008.

POS-WED-175

IMMUNOHISTOCHEMICAL AND FUNCTIONAL STUDY OF GALANIN IN THE RAT ROSTRAL VENTROLATERAL MEDULLA (RVLM)

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Purpose: The neuropeptide galanin (GAL) occurs throughout the brain, including in RVLM, which contains pivotal neurons for tonic and reflex control of arterial pressure. Functional studies suggest a role for galanin in central cardiovascular regulation. However, the cardiovascular effects of injecting GAL into RVLM have not been extensively studied and the relationship of GAL-immunoreactive axons to barosensitive RVLM neurons is unknown. This study aimed to investigate the presence and direct action of GAL in RVLM. **Methods:** Cannulae were implanted into femoral arteries and veins of isoflurane-anesthetized Sprague Dawley rats (n= 6) for recording arterial pressure (MAP) and infusing drugs, respectively. The next day, conscious rats were treated with hydralazine (HDZ, 2mg/ml) or saline and perfused 2 hours later. Sections of brainstem were stained for GAL, Fos and PNMT with avidin-biotin-peroxidase. To investigate GAL's cardiovascular effects, it was micro-injected into the RVLM (20 nl or 40 nl of 1 ng/nl of aCSF) of isoflurane-anesthetized rats (n= 5) **Results:** GAL-immunoreactivity occurred in neurons that lay close to PNMT-immunoreactive neurons. HDZ infusion produced significant hypotension (67±5 mmHg vs 110±10) and induced Fos-immunoreactivity in many PNMT neurons in the RVLM. Very few Fos/PNMT neurons were found after saline infusion. GAL-immunoreactive terminals closely apposed about half of the barosensitive C1 neurons. Microinjection of 40ng GAL consistently decreased MAP to about 25 mmHg below baseline; vehicle microinjection did not change MAP. **Conclusion:** This study shows that GAL acts as a depressor substance when injected into the RVLM and suggests that GAL-immunoreactive neurons directly innervate barosensitive C1 RVLM neurons.

POS-THU-176

CLOSE ARTERIAL LEPTIN INFUSION SELECTIVELY INHIBITS A SUB-POPULATION OF RVLM NEURONS AND SPLANCHNIC SYMPATHETIC NERVE ACTIVITY

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Purpose: Evidence is emerging for an interactive relationship between the gastric (non-adipose) source of leptin and the gastrointestinal hormone cholecystokinin (CCK). The aim of this study was to examine the significance of these signals in cardiovascular regulation. **Methods:** The effect of baroreflex activation, CCK administration (1-4 µg/kg, i.v.) and close arterial (coeliac) infusion of leptin (10-30 µg/kg) on (i) arterial pressure (AP), (ii) heart rate (HR), (iii) firing rate (FR) of presympathetic vasomotor neurons in the rostroventrolateral medulla (RVLM), and (iv) splanchnic/lumbar sympathetic nerve discharge (SND), was examined in isoflurane anesthetised, paralysed, male Sprague-Dawley rats. **Results:** Within 5 minutes of administration, close arterial leptin infusion significantly decreased the FR of CCK-sensitive (i.e. inhibited) RVLM neurons (-28 ± 5 %; $P < 0.01$; n=10) but not of CCK-insensitive/activated neurons (8 ± 6 %; $P > 0.05$; n=6), when compared to saline controls (-1 ± 2 %; n=11). In contrast, intravenous leptin had no effect on the FR of CCK-sensitive neurons (2 ± 4 %; $P > 0.05$; n=5). Close arterial leptin administration significantly decreased AP (-13 ± 3 mmHg; $P < 0.05$; n=16) compared to close arterial saline (-4 ± 1 mmHg; n=11) and intravenous leptin (3 ± 1 mmHg; n=5). In contrast, HR remained unaffected. In separate experiments, splanchnic SND was significantly inhibited by close arterial leptin infusion (-9 ± 3 %; $P < 0.05$; n=7) compared to close arterial saline infusion (2 ± 3%; n=5), whereas lumbar SND was unaffected. **Conclusion:** Leptin acting within the gut may exert its sympathetic effects via inhibition of a sub-population of CCK-sensitive RVLM neurons, implicating it in gastrointestinal circulatory control.

POS-WED-177

PROPERTIES OF NEURONS IN THE ROSTROVENTROLATERAL MEDULLA THAT ARE ACTIVATED BY NEUROGLUCOPRIVATION

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Purpose: The sympathetic nervous system alters glycaemia via mechanisms that include the release of adrenaline. In this study we have tested the hypothesis that neuroglucoprivation activates a sub-population of medullospinal neurons in the rostroventrolateral medulla (RVLM) and that the RVLM influences blood glucose levels. **Methods:** In isoflurane-anesthetized, paralysed male Sprague-Dawley rats, we recorded the discharges of RVLM spinally-projecting neurons along with arterial blood pressure and heart rate. RVLM medullospinal neurons were identified by electrical stimulation of the thoracic spinal cord. Spinally-projecting neurons were tested for sensitivity to (i) baroreceptor stimulation (abdominal aortic occlusion), (ii) cholecystokinin (CCK, 4 µg/kg, i.v.; abdominal vagal sympathoinhibitory reflex), (iii) phenylbiguanide (PBG, 10 µg/kg, i.v.; von Bezold-Jarisch reflex) and (iv) neuroglucoprivation (2-deoxyglucose, 2-DG; 300 mg/kg, i.v.). **Results:** RVLM medullospinal neurons (n=20) displayed sensitivity to baroreceptors (17/20), PBG and had variable sensitivity to CCK. Some (8/16) were modestly inhibited by 2-DG. These neurons had spinal axonal conduction velocities similar to those reported previously (0.4-6.4 m/s). In contrast, we characterised a small population of neurons (n=3) that were prominently activated by 2-DG. These mostly had slow conducting spinal axons and were insensitive to baroreceptor activation. In a separate series of experiments under urethane anaesthesia (1.4 g/kg, i.v.), bicuculline (100 pmol/100 nl) was microinjected bilaterally into the RVLM. Along with the expected increase in arterial blood pressure, blood glucose concentration rose from 4.7±0.4 mM to 15.1±1.0 mM ($P<0.05$; n=4). **Conclusion:** Neurons that are activated by 2-DG are likely to be those that control adrenaline release and produce hyperglycaemia. This population appears to be distinct from those classically described as RVLM presympathetic vasomotor neurons.

POS-THU-178

CARDIOVASCULAR AND LOCOMOTOR EFFECTS OF OREXIN A ADMINISTERED INTRACEREBROVENTRICULARLY AND INTRATHECALLY IN THE CONSCIOUS RAT

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Orexin A (or hypocretin 1) is a neuropeptide involved in the regulation of arousal. Orexinergic projections, which innervate many regions in the brain, extend as far down as the spinal cord where they target sympathetic preganglionic neurons (SPNs). This projection to SPNs is dense at upper thoracic levels (T1-T2) but sparse below. Intracerebroventricular injection of orexin A in the conscious rat produces increases in heart rate (HR) and mean arterial pressure (MAP) associated with locomotor activity. The effect of intrathecal injection at thoracic levels in the conscious rat is not known, although increases in HR and MAP have been reported under anaesthesia from T2-T3. **Purpose:** Compare intracerebroventricular and intrathecal effects of orexin A on HR, MAP and locomotor activity in the conscious rat. **Method:** Animals were implanted with radio-telemetric probes and with either a cannula in the lateral ventricle or an intrathecal catheter placed at T2-T3 or T8-T9. Orexin A (0.3 nmol in 10 µl) was compared to vehicle. **Results:** As expected, orexin A into the lateral ventricle evoked a significant increase in HR, MAP and locomotor activity ($n=12$, $p<0.05$). Intrathecal administration of orexin A at T2-T3 also evoked a significant increase in HR ($n=4$, $p<0.03$), but had no effect on MAP and activity ($n=4$, $p>0.05$). At T8-T9, orexin A had no effect on any of the variables ($n=6$, $p>0.08$). **Conclusion:** The main effect of orexin injected at thoracic levels appears to be on SPNs that preferentially control heart rate rather than blood pressure. This is consistent with the greater density of orexinergic projections to upper thoracic levels, which contain most of the cardiac SPNs but few vasomotor SPNs.

POS-WED-179

THE OREXIN RECEPTOR ANTAGONIST ALMOREXANT REDUCES RAT DIURNAL CARDIOVASCULAR RESPONSES TO PSYCHOLOGICAL, BUT NOT PHYSICAL STRESS

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The neuropeptides orexins/hypocretins are implicated in arousal and the maintenance of wakefulness. Orexin neurons may also regulate some biological responses to stress, in part because of their location in the hypothalamic defence region. **Purpose:** Test the role of orexins in the expression of cardiovascular and behavioural responses to different forms of stress and arousal. **Methods:** Almorexant, a dual orexin receptor antagonist, was administered orally by gavage at 0, 100 and 300 mg/kg doses, 2.5 hr prior to testing. Heart rate (HR), mean arterial pressure (MAP) and activity were recorded by telemetry. **Results:** When tested during the diurnal rest phase, Almorexant had no effect on baseline HR, MAP and Activity (n=32) but reduced all three variables during exploration of an unfamiliar environment (n=7). In conditioned fear stress, the MAP response was significantly blunted (n=10). A reduction of the sympathetic component of the associated HR response (revealed after atropine treatment) was also observed. In contrast, Almorexant had no effect on any of the three variables during restraint and cold exposure (n=8). Finally, when tested on spontaneous foraging behaviours during the nocturnal active phase, Almorexant significantly reduced all three variables (n=10). **Conclusion:** Consistent with previous c-Fos expression studies, the present results show that orexins are implicated in the diurnal cardiovascular response to some but not all forms of stress. Orexin appears to be required when the stressor is psychological or centrally generated, such as during conditioned fear and exploration, but not when the stressor is physical and immediate, such as during restraint and cold exposure.

POS-THU-180

EFFECTS OF VOLUNTARY EXERCISE ON CARDIAC RESPONSES TO STRESS AND ON CARDIAC EXCITABILITY

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Purpose: To study effects of voluntary exercise on cardiac responses to acute psychological stressors and on myocardial contractile and excitable properties. **Methods:** Effects of 8-week voluntary exercise were studied in Hooded Wistar rats. Group1 rats (Runners, n=8) were in the cages with attached running wheels. Group2 rats (Controls, n=8) were in normal cages. After 8 weeks of exercise (or similar control period), telemetric ECG transmitters were implanted. Animals were subjected to restraint and, on different days, to social defeat. In subsequent acute experiments, we compared LVP, LVdP/dt, effects of autonomic blockade, of beta-adrenoreceptor stimulation, of afterload changes and of proarrhythmic agent aconitine in runners vs. controls. **Results:** Basal HR of runners (356±19 bpm) was lower compared to controls (384±10 bpm; $p<0.05$). In runners, high-frequence power of HRV was elevated compared to non-runners. There was no differences between groups with regard to tachycardic responses to restraint (peak tachycardia 492±18 bpm in runners vs. 491±14 bpm in non-runners) or social defeat (peak tachycardia 507±11 bpm in runners vs. 500±13 bpm in non-runners), in the intrinsic heart rate, or in the weight of the heart. In anesthetized animals, there were no between-group differences in AP, HR, LVP, LVdP/dT, LVEDP or in sensitivity of these indices to isoproterenol. There was a significant difference in the minimal dose of aconitine that provoked first ectopic beat (109±33 µg runners vs. 33±8 µg controls). **Conclusions:** Modest voluntary exercise (without cardiac hypertrophy) possesses antyarrhythmic effects; this is associated with elevated respiratory sinus arrhythmia.

POS-WED-181

ACTIVATION OF A PATHWAY FROM THE HYPOTHALAMIC PVN TO THE NTS DURING AIRPUFF STRESS

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Purpose: The nucleus of the solitary tract (NTS) is an essential component of the central pathways mediating cardiovascular reflexes. A previous study in this laboratory showed that neurons of the NTS are active (express Fos) during airpuff, a highly arousing stimulus that activates the cardiovascular system. This study combined Fos with a retrograde tracer targeted at the NTS to identify central neurons that may modulate the NTS during airpuff stress. **Method:** In a preliminary operation under isoflurane anaesthesia, iontophoresis was used to microinject the retrograde tracer, CTB, into the NTS of male Sprague-Dawley rats. One week later the rats were either exposed to a 300kPa airpuff (30 mins, n=3) or not exposed (control, n=3). Two hours later, the rats were deeply anaesthetised and perfused, and the brains removed for double-immuno-processing for CTB and Fos. **Results:** Dense retrograde CTB labelling from the NTS was found in the paraventricular hypothalamus (PVN) and central nucleus of the amygdala, and moderate labelling in the perifornical and lateral hypothalamus, Killiker-Fuse nucleus and rostral ventrolateral medulla. Although Fos expression was evoked by airpuff in most of these regions, only the PVN contained a significant number of Fos-CTB double-labelled cells (13.6% of CTB-labelled cells following airpuff, compared with 0.5% for control, $p<0.05$). **Conclusion:** Airpuff stress activates neurons in the PVN that project to the NTS. The pathway from the PVN to the NTS may mediate the modulation of the baroreceptor reflex that is known to occur during psychological stress (Kanbar R et al, Am J Physiol. 292: R362-R367, 2007).

POS-THU-182

CARDIORESPIRATORY RESPONSE EVOKED BY THE DORSAL PAG IN THE MIDBRAIN IS MEDIATED VIA THE DORSOMEDIAL HYPOTHALAMUS

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Purpose: The midbrain periaqueductal grey (PAG) and the dorsomedial hypothalamus (DMH) both play crucial roles in mediating physiological responses to acute psychological stress. Activation of neurons in both the dorsal PAG and DMH causes increases in blood pressure, heart rate, sympathetic activity and respiratory activity, similar to the stress responses. There are conflicting observations as to whether the descending cardiovascular pathway from the DMH includes a synapse in the dorsal PAG (1,2). On the other hand, there is an ascending projection from the dorsal PAG to the DMH. In the present study, we therefore tested the hypothesis that the pathways mediating the sympathoexcitatory and respiratory responses evoked from the dorsal PAG are mediated via the DMH. **Methods:** Arterial pressure, heart rate (HR), renal sympathetic nerve activity (RSNA), and phrenic nerve activity (PNA) were recorded in rats (n=6) anaesthetized with urethane. **Results:** Microinjection of DL-homocysteic acid (5 nmol) into the dorsal PAG caused in increases in mean arterial pressure (MAP), HR, RSNA and PNA burst rate of 22±2 mmHg, 73±16 bpm, 117±39%, and 45±12 bursts per min, respectively. Bilateral injections of muscimol (1 nmol in 50 nl in each site) into the DMH greatly attenuated the increases in MAP, HR, RSNA and PNA burst rate (to 25±3%, 5±6%, 12±5%, and 9±4% of the respective control responses). **Conclusion:** The results indicate that the cardiorespiratory response evoked from the dorsal PAG in the midbrain is mediated by neurons in the DMH. 1) da Silva et al, *Brain Res.* 984: 206-214, 2003 2) Horiuchi et al, *Proceedings of the ANS* vol. 18: p81, 2008.

POS-WED-183

DIFFERENTIAL CONTROL OF SYMPATHETIC VASOMOTOR AND RESPIRATORY FUNCTIONS BY THE DORSAL PERIAQUEDUCTAL GREY IN THE MIDBRAIN

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Purpose: Neurons in the midbrain periaqueductal grey (PAG) mediate physiological responses to stress. Activation of neurons in the dorsal PAG causes increases in blood pressure, heart rate, sympathetic activity and respiratory activity, similar to the stress responses. In the present study, we tested whether PAG neurons that evoked increases in renal sympathetic nerve activity (RSNA) and in respiratory rate (RR) have similar or different locations within the dorsal PAG. **Methods:** Arterial pressure, heart rate (HR), RSNA and phrenic nerve activity were recorded in rats (n=9) anaesthetized with urethane. **Results:** Microinjections of DL-homocysteic acid (5.0 nmol) into the dorsal PAG caused large increases in RSNA (117±39% of baseline) and RR (42±13%), but there was a negative correlation between the magnitudes of the RSNA and RR responses (r = -0.60). Much smaller microinjections (750 pmol) into 54 sites in 3 rats evoked moderate or large (>20%) increases in RSNA only at 17 sites, in RR only at 5 sites, and in both RSNA and RR at 7 sites. Sites evoking the largest respiratory responses were located lateral to those evoking the largest RSNA responses. **Conclusion:** The results indicate that the sympathetic and respiratory responses evoked from the dorsal PAG arise from activation of topographically distinct groups of neurons, as has been described previously for the dorsomedial hypothalamus (1). 1) Tanaka and McAllen *Am J Physiol Regul Integr Comp Physiol.* 2008 294(2):R477-86.

POS-THU-184

AMYGDALA MEDIATES SYMPATHETIC CUTANEOUS VASOCONSTRICTOR ALERTING RESPONSES (SCVARS) IN RATS: RELEVANCE OF 5HT2A RECEPTORS

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Purpose: Sympathetic neuronal outflow to cutaneous vascular beds is selectively activated by salient events, so that cutaneous blood flow is characterized by sudden alerting-related falls to near zero levels (SCVARs, Sympathetic Cutaneous Vasoconstrictor Alerting Responses [1]). Our previous work indicates that amygdala contributes to coordinate SCVARs in rabbits [2]. In the present study we first investigated the involvement of amygdala in the control of SCVARs in rats, and then studied the possible neurotransmitters modulating SCVARs in the amygdala. **Methods:** Male Sprague Dawley rats (250-350g) were chronically implanted with tail artery Doppler flow probes and stainless steel guide cannulae directed bilaterally towards the central amygdala. After a one-week the rats were either injected with $GABA_A$ receptor agonist muscimol 1 nmol/100nl (n=13) or the vehicle (n=12) into the central amygdala and 30 min later they were subjected to five standard alerting stimuli. SCVAR index was calculated for each rat [1]. Similar experiments were conducted in another group of rats (n=8) following central amygdala injection of 5HT2A receptor antagonist SR 46349B in three different doses. **Results:** After muscimol SCVAR index was 20.96±5.14%, compared with 83.18±3.07% after vehicle. SR 46349B 1, 5, 25 nmol/200nl inhibited SCVARs dose dependently. Log-dose regression ($F_{(1,23)} = 18.519$, $P=0.0003$). **Conclusion:** Amygdala is involved in coordinating SCVARs in rats. 5HT2A receptors contribute to SCVAR modulatory effect of the amygdala. [1] Psychopharmacology 2005, 181, 518-28. [2] AmJPhysiol., 1997, 272, 208-16.

POS-WED-185

THE PROJECTION FROM THE CENTRAL NUCLEUS OF THE AMYGDALA TO THE VENTROLATERAL PERIAQUEDUCTAL GRAY IS GABAERGIC

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Most current models of conditioned fear propose that upon exposure to a conditioned stimulus, the main output nucleus of the amygdala, the central nucleus (CeA), is activated. This results in the activation of the ventrolateral periaqueductal gray (VLPAG) via a direct projection, which induces freezing. However, it is becoming increasingly clear that projections arising from the CeA are GABAergic. **Purpose:** To determine the proportion of amygdaloid projections to the VLPAG which contain GABA. **Methods:** The retrograde tracer cholera toxin subunit B (CTB) was injected into the caudal VLPAG of rats (n = 2), and GAD-67 mRNA was revealed by in situ hybridisation. Single and double labelled cells were counted throughout the amygdala. **Results:** In both rats, CTB immunoreactive cells were found in the CeA (48% and 56% of the total number of CTB immunoreactive cells in the amygdala), dorsal medial amygdala (MeD; 18% and 16%), ventral medial amygdala (MeV; 11% and 14%) and basomedial amygdala (BMA; 8% and 12%). The percentage of CTB immunoreactive neurons that contained GAD-67 mRNA was 93% and 95% in CeA; 32% and 52% in MeD; 17% and 11% in MeV; and 18% and 31% in BMA. **Conclusion:** The projection from the CeA to the VLPAG is almost entirely GABAergic. This raises the question of how the CeA can induce activity in VLPAG neurons during conditioned fear. One possibility is that GABAergic output neurons are tonically active and *inhibited* during fear. Another possibility is that they are activated during fear but synapse onto GABAergic VLPAG neurons. Either way, VLPAG activation may occur by disinhibition.

POS-THU-186

VENTROLATERAL PERIAQUEDUCTRAL GRAY PROJECTIONS TO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS DO NOT POSSESS α2A AND α2C-ADRENOCEPTORS

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Purpose: Chronic constriction injury (CCI) of the sciatic nerve results in hyperalgesia and allodynia in all rats, however in ~30% of CCI rats there are increased corticosterone levels, which accompany other CCI evoked behavioural disabilities. Tissue injuries evoke increased activity in the medullary, noradrenergic, A1 cell group. Almost all of the A1 neurons project to the paraventricular nucleus of the hypothalamus (PVH) which regulates corticosterone release, 80% of the A1 cells also project to the ventrolateral periaqueductal gray (vlPAG). This raises the question of whether noradrenergic inputs to the vlPAG activate a second source of afferent drive onto the PVH. To address this possibility we investigated whether PVH projecting, vlPAG neurons possess α2a and α2c-adrenoreceptors. **Methods**: Male Sprague-Dawley rats (N=20) were anaesthetised and the retrograde tracer, Fluorogold (FG) was injected iontophoretically into the PVH. Standard fluorescence immunohistochemical techniques were used to label α2a and α2c-adrenoreceptors in serial sections of the PAG. Single (FG+) and double-labelled (FG+ and α2a or α2c-adrenoreceptors) vlPAG neurons were quantified. **Results**: The projection from the vlPAG to the PVN is primarily ipsilateral (~80% ipsilateral), more than half of these cells being located in the caudal-most region of the vlPAG. (-8.3 to –8.8 mm from bregma). There were no double labelled neurons in the vlPAG, despite clear evidence of α2a and α2c-adrenoreceptor immunoreactivity on FG- cells. **Conclusion**: While vlPAG neurons project into the PVN and undoubtedly play a role in the regulation of corticosterone release, it is unlikely that a direct, post-synaptic action of noradrenaline plays a role in the actions of vlPAG neurons in this response.

POS-WED-187

PERIAQUEDUCTAL GREY TO PARAVENTICULAR NUCLEUS OF THALAMUS PROJECTIONS AS A POSSIBLE PATHWAY FOR INJURY EVOKED CHANGES IN HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FUNCTION

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Purpose: It has been shown that neurons of the posterior paraventricular nucleus of the thalamus (pPVTh) inhibit the reactivity of the hypothalamic pituitary adrenal (HPA) axis during chronic, but not acute stress. Using retrograde anatomical tracing techniques we have shown, in stress naïve rats, that the pPVTh receives a significant projection from neurons located in the ventrolateral column of the periaqueductal grey (vlPAG). Furthermore, using double-labeling techniques we have shown that pPVTh projecting vlPAG neurons do not possess glucocorticoid receptors, suggesting this pathway is not directly modulated by systemic corticosterone levels. In rats, chronic stressors have been shown to increase levels of the neuropeptide cholecystokinin (CCK) in the PAG. In particular, we have shown that chronic constriction injury (CCI) of the sciatic nerve significantly increases the density of CCK-immunoreactive terminals/varicosities in the vlPAG of rats, which show HPA-axis dysfunction. The aim of this study was to determine the spatial relationships of CCK-IR terminals with pPVTh projecting vlPAG neurons. **Method**: In 28 rats, retrograde tracer was placed into the PVTh and double-labelling studies used to reveal the location of PVTh projecting vlPAG neurons and CCK-IR. **Results**: Analysis revealed that 29.2% (±.94) of vlPAG -posterior PVTh neurons were directly opposed by CCK-IR terminals, whereas, only 10.9% (±1.2) of vlPAG - anterior PVTh projecting neurons were directly opposed by CCK-IR terminals ($p<0.01$). **Conclusion**: These data suggest that in the un-injured (stress naïve) state, CCK likely plays a role in regulating the vlPAG-pPVTh pathway. The effects of injury on this spatial relationship can now be investigated as next experimental step.

POS-THU-188

RATS WITH PAIN AND DISABILITY FOLLOWING SCIATIC NERVE INJURY HAVE INCREASED NUMBERS OF NEURONS EXPRESSING CHOLECYSTOKININ MRNA IN THE VENTROLATERAL PERIAQUEDUCTAL GRAY

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Purpose: Chronic constriction injury (CCI) of the sciatic nerve evokes behavioural and physiological dysfunction in only a sub-population of rats (~30%) despite all animals displaying the sensory changes characteristic of neuropathic pain. GeneChip and RT-PCR analyses of the periaqueductal grey region (PAG) of these rats revealed a specific increase in CCK mRNA in rats with disabilities. The aim of these experiments was to localise anatomically the cells in which this selective gene up-regulation was occurring. **Methods**: Male Sprague-Dawley rats (N=36) were behaviourally categorized into three groups on the basis of altered social interactions following CCI. Serial coronal sections of the PAG were obtained from rats with pain and disability (N=6), rats with pain and transient disability (N=6) and rats with pain alone (N=6). In situ hybridisation techniques using a 35S oligonucleotide probe were used in combination with fluorescence immunohistochemistry to locate cells expressing CCK mRNA and tyrosine hydroxylase (TH-IR) in six equidistant sections through the rostro-caudal extent of the PAG. **Results**: CCK mRNA was detected in cells of the lateral and ventrolateral (vlPAG) columns of the PAG in all rats. In rats with pain and disability, there was a 90% increase in the number of vlPAG neurons expressing CCK mRNA compared with the other CCI rats. In addition, almost all cells in which CCK mRNA was detected also contained TH-IR. **Conclusions**: CCI triggers a select increase in CCK mRNA in the vlPAG of a subpopulation of rats. The functional significance of this up-regulation is an important next question.

POS-WED-189

A 90 MINUTE ULTRADIAN RHYTHM IN BROWN ADIPOSE TISSUE IS HIGHLY CORRELATED WITH SIMILAR RHYTHMS IN BRAIN TEMPERATURE AND HIPPOCAMPAL THETA EEG

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Purpose: Brown adipose tissue (BAT) temperature increases in an ultradian manner approximately every 90 min in rat[1]. Since brain temperature has also been shown to exhibit an ultradian rhythm, we investigated the relationship between BAT and brain temperature, and determined whether ultradian BAT temperature were related to similarly timed variations in the proportion of theta (5-8 Hz) power in the hippocampal EEG. **Methods:** Rats (Sprague Dawley, 330-450 g) were instrumented with thermistors under isoflurane anesthesia for continuous measurement of BAT, brain and body temperatures. In some rats EEG electrodes were also positioned in the dorsal hippocampus. Wires from probes were connected to a headpiece fixed to the skull. At least one week later, conscious unrestrained rats were placed in a quiet constant temperature (24°C) environment with 12 hour light/dark cycle. **Results:** During the dark (active) phase, BAT temperature increased by 1.1±0.1°C every 82±3 min (129 occasion in 9 rats), highly correlated with increases in brain temperature (R=0.92±0.01, 14 occasions in 9 rats), and in the proportional power of hippocampal theta-rhythm (R=0.59±0.03, 45 occasions in 7 rats,). The hippocampal theta-rhythm became prominent preceding the onset of iBAT thermogenesis (6.3±0.6 min, n=7). The onset of an increase in BAT temperature preceded brain temperature onset (2.6±0.8 min, 128 occasions in 9 rats) **Conclusion:** Our results demonstrate that sudden increase in heat production by BAT makes a major contribution to ultradian rhythm in brain temperature. We propose that this ultradian metabolic rhythm facilitates cognitive function in a manner that preserves energy stores. [1] Y.Ootsuka, ANS 2008 Proceedings, POS-TUE-065.

POS-THU-190

WHAT FUNCTIONS DO VASOMOTOR GANGLION CELLS PERFORM IN VIVO?

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Purpose:. To understand the functional relevance of synaptic processing by vasomotor ganglion cells. **Methods:** In 17 urethane-anaesthetized (1-1.5 g/kg, i.v.) artificially ventilated rats, we made intracellular recordings of spontaneous activity in lumbar sympathetic ganglion cells (L3/4) with intact central connections. **Results:** In 38/42 stably recorded cells with ongoing activity, both spikes and subthreshold EPSPs showed strong cardiac rhythmicity, demonstrating barosensitivity. These cells were therefore of vasomotor type. Hyperpolarizing the membrane revealed that they received synaptic inputs with a range of amplitudes, including a single 'strong' (> 20 mV) unitary EPSP in most cells. At resting potential, the membrane trajectory preceding spikes showed that, for the population, about one third of all spontaneous action potentials were attributable to 'strong' synaptic inputs. Most other spikes were driven by unitary EPSPs of lesser amplitude, slower rise time and greater quantal variability. Only a minority were driven by summation of subthreshold EPSPs. Calculations from the distribution of EPSP amplitudes indicated that in most ganglion cells, spike firing rate was a continuously variable function of resting membrane potential. **Conclusions:** 'Strong' preganglionic inputs always trigger postganglionic action potentials, providing 'obligatory' ganglionic transmission. Weaker inputs may fall above or below cell threshold, and thus provide potentially variable ganglionic transmission. Factors that change ganglion cell excitability (circulating hormones, modulatory synaptic inputs) may therefore have sensitive control over ganglionic throughput and vascular tone. The vasomotor ganglionic synapse thus show little capacity for integration (synaptic summation) but provides a continuously variable gain control.

POS-WED-191

ROLES OF TWO PREOPTIC CELL GROUPS IN TONIC AND FEBRILE CONTROL OF RAT TAIL SYMPATHETIC FIBRES

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Heat dissipation from the skin is reduced both in a cold environment and in a fever, and is regulated by sympathetic vasoconstrictor nerves under the control of the brain. The preoptic area is considered to be essential for thermoregulation and febrile responses, but there is limited information regarding the regulation of cutaneous vasomotor nerves. We made recordings of sympathetic nerve activity (SNA) of cutaneous vasoconstrictor fibres supplying tail in 30 urethane-anaesthetised rats. Inhibition of neurons in the preoptic area by microinjection of GABA (300 mM, 15-30 nl) increased tail SNA. Two distinct GABA-sensitive preoptic regions were identified; the rostromedial preoptic region (RMPO) including the organum vasculosum of the lamina terminalis (OVLT) and the median preoptic nucleus (MnPO), and the caudal preoptic region (CPO). These two regions showed similar sensitivity to GABA. Next we focused on the two preoptic regions and investigated their sensitivity to prostaglandin E_2 (PGE_2), which is the final humoral mediator of fever. Microinjections of PGE_2 (0.2 and 1 ng, 15 nl) into GABA-sensitive sites in the RMPO induced a rapid increase in tail SNA followed by a rise in core temperature, but the same treatments to GABA-sensitive sites in the CPO was ineffective. These results suggest that neurons in both the RMPO and the CPO are tonically active and dually provide tonic inhibitory drives to tail vasoconstrictor systems. The RMPO is probably a source for heat conservation mechanisms during fever, but the CPO is unlikely to mediate such effects.

POS-THU-192

MODULATION OF SKIN SYMPATHETIC NERVE ACTIVITY AND PROMOTION OF CARDIAC RHYTHMICITY BY SINUSOIDAL GALVANIC VESTIBULAR STIMULATION IN HUMAN SUBJECTS

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Purpose: We have previously shown that sinusoidal GVS, a means a selectively altering vestibular afferents without affecting other inputs can cause partial entrainment of muscle sympathetic nerve activity (Bent et al., 2006). Given that motion sickness causes sweating and pallor, we tested the hypothesis that sinusoidal GVS also entrains skin sympathetic nerve activity (SSNA), but that the optimal frequencies are closer to those associate with postural changes (0.2 Hz). **Methods:** SSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 11 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 200 cycles) was applied to the mastoid processes at frequencies of 0.2, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 Hz. **Results:** All subjects reported strong postural illusions of 'rocking in a boat' or 'swaying in a hammock'. Vestibular modulation of SSNA occurred at all frequencies but was stronger at 0.2 Hz (81.5±4.0 %) and significantly weaker at 2.0 Hz (63.2 ±5.4%; $p<0.01$). Conversely, cross-correlation analysis revealed that cardiac modulation of SSNA was stronger at 0.8 Hz (86.2±2.0 %) and weaker at 0.2 Hz (69.3±8.3%) or 0 Hz (66.0±6.4%). **Conclusions:** These observations demonstrate that low-frequency vestibular inputs have a modulatory affect on SSNA. Additionally, sinusoidal GVS increases cardiac rhythmicity of cutaneous sympathetic neurones, which is normally weak. **Reference:** Bent LR, Bolton PS & Macefield VG, Modulation of muscle sympathetic bursts by sinusoidal galvanic vestibular stimulation in human subjects, Exp Brain Res 2006 174: 701-711.

POS-WED-193

FREQUENCY-DEPENDENT MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY BY SINUSOIDAL GALVANIC VESTIBULAR STIMULATION IN HUMAN SUBJECTS

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Purpose: Muscle vasoconstrictor neurones play an important role in maintaining blood pressure during postural challenges, but the relative contributions of baroreflexes and vestibular inputs are poorly understood. We have previously demonstrated that selective modulation of vestibular inputs, via sinusoidal galvanic vestibular stimulation (GVS) delivered at 0.5-0.8 Hz, can cause partial entrainment of muscle sympathetic nerve activity (MSNA; Bent et al., 2006). Given that we had seen interaction between the dynamic vestibular input and the normal cardiac-locked MSNA rhythm, we tested the hypothesis that frequencies of GVS remote from the cardiac frequency would cause a greater modulation of MSNA than those around the cardiac frequency. **Methods:** MSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 11 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 200 cycles) was applied to the mastoid processes at frequencies of 0.2, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 Hz. **Results:** In all subjects the stimulation evoked robust vestibular illusions of "rocking in a boat" or "swinging from side to side." Cross-correlation analysis revealed a cyclic modulation of MSNA at all frequencies, with the modulation index being similar between 1.1 Hz (78.5±3.7 %) and 2.0 Hz (77.0±4.3 %). However, vestibular modulation of MSNA was significantly stronger at 0.2 Hz (93.1±1.7 %) and significantly weaker at 0.8 Hz (67.2±1.8 %). **Conclusions**: Our data demonstrate that low-frequency changes in vestibular input (0.2 Hz), such as those associated with postural changes, preferentially modulate MSNA. Furthermore, our data suggest that vestibular inputs compete with the stronger baroreceptor inputs operating at the cardiac rhythm (~0.8 Hz), with vestibular modulation of MSNA being greater when this competition with the baroreceptors is reduced. **Reference:** Bent LR, Bolton PS & Macefield VG, Modulation of muscle sympathetic bursts by sinusoidal galvanic vestibular stimulation in human subjects, Exp Brain Res 2006 174: 701-711.

POS-THU-194

MAPPING PUDENDAL-LUMBAR SPINAL-PELVIC PATHWAYS CONTROLLING FEMALE REPRODUCTIVE TRACT IN GUINEA-PIGS

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Purpose: The pudendal-spinal-pelvic pathway controlling blood flow to the uterus is essential for normal reproductive and sexual activity. Our previous work showed that this pathway can be activated at sacral spinal levels in isolated preparations. But, it is not clear whether there is a functional spinal pathway from pudendal sensory nerves to uterine vasodilator pathways projecting from the lumbar cord to the pelvic ganglia. **Methods**: Female guinea-pigs (200-250g) were anaesthetised with 50% urethane (ip). In isolated preparations perfused via the abdominal aorta, electrical pulses were applied to the central end of the pudendal nerves and suction electrodes were used to test for evoked compound action potentials in the hypogastric and L3 lumbar splanchnic nerves in the absence or presence of the GABAA receptor antagonist, bicuculline (10μM). **Results**: Single or rythmic (200Hz) electrical stimulation of pudendal nerves (0.3ms, 50V) evoked small compound action potentials in hypogastric (44 ± 3μV; 64 ± 8μV; 77 ± 11μV for 1, 5 and 10 pulses) and L3 lumbar splanchnic nerves (53± 6μV; 66 ± 8μV; 81 ± 7μV for 1, 5 and 10 pulses). After addition of bicuculline (10μM), the responses were significantly increased to 71 ± 7μV; 96 ± 6; 106 ± 7 in hypogastric nerves and to 76 ± 5μV; 100 ± 7; 116 ± 10 for 1, 5 and 10 pulses in splanchnic nerves (n=8; $p<0.05$). **Conclusion**: In addition to the sacral pathway, there is a functional spinal pathway activated from the pudendal nerve that leaves the lumbar cord to synapse in pelvic ganglia and regulate the uterus and its vasculature. This pathway is modulated by $GABA_A$ receptors.

POS-WED-195

INTERLEUKIN-6 MEDIATED ACTIVATION OF ERK1/2 AND STAT3 IN ADRENAL MEDULLARY CHROMAFFIN CELLS

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Bunn S.J., Carman F. and Douglas S.A. The Centre for Neuroendocrinology and Dept of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand. **Purpose:** A bi-directional relationship exists between the immune and neuroendocrine systems which may contribute to a number of pathologies, most notably those relating to stress. The catecholamine secreting chromaffin cells of the adrenal medulla are important players in the response to stress. We report here on the ability of the immune-derived cytokine interleukin-6 (IL6) to signal to these neuroendocrine cells. **Methods:** Bovine chromaffin cells were isolated from the gland, purified by differential plating and cultured on collagen-coated wells. Cells were washed twice with a physiological salts solution and then incubated with IL-6 (1nM) for various periods of time. Standard immunoblotting procedures were used to detect activated proteins. **Results:** IL6 caused a transient increase in ERK1/2 phosphorylation, maximal after about 15 -30 mins ($p<0.01$). This enhanced ERK activation was accompanied by a selective increase in the ser-31, but not ser-19 or ser-40 phosphorylation, of tyrosine hydroxylase, the rate-limiting catecholamine synthesizing enzyme ($p<0.05$). This phosphorylation may have implications for regulation of tyrosine hydroxylase activity. In addition to ERK activation IL6 stimulated the tyrosine phosphorylation and nuclear localization of signal transducer and activator of transcription (STAT)3 in approximately 50% of the cells ($p<0.001$). IL6 stimulation also increased the serine phosphorylation of STAT3 ($p<0.05$) through an ERK1/2 dependent (PD 98,059 inhibited) pathway. **Conclusions:** These data provide evidence that the neuroendocrine chromaffin cells are sensitive to IL6, suggesting a pathway linking immune-derived signals to the adrenal medullary stress response. Importantly this IL6 response has the potential to regulate both acute enzyme activity and gene expression.

POS-THU-196

SORTILIN IS EXPRESSED BY HUMAN NATURAL KILLER CELLS AND MEDIATES PRONGF INDUCED CELL DEATH

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Purpose: It is well established that nerve growth factor has important functions in the immune system. Recent data indicates that pro-forms of neurotrophins mediates cell death in the nervous system by binding receptor complexes consisting of the neurotrophin receptor p75 and sortilin. It is likely that pro-neurotrophins causes similar responses in the immune system. In this study we examined sortilin expression in immune cells and evaluated its role in proNGF-mediated cell death in human natural killer cells (NK). **Methods**: Flow cytometry and western blot were used to examine expression of neurotrophin receptors by leucocytes in peripheral blood. NK cells were isolated using negative selection and were treated with mature NGF or cleavage resistant proNGF over various time periods and apoptosis and necrosis examined using AnnexinV and propidium iodide staining. Apoptotic signaling was also examined by western blots. **Results**: Multiparameter flow cytometry demonstrated that sortilin was expressed by NK cells from all donors (n=8). Subsequent analysis showed that NK cells also expressed p75 and TrkA (n=4). NK cells treated with proNGF or mature NGF underwent apoptosis and necrosis (n=3). Preliminary experiments suggest that this proNGF-induced apoptosis is inhibited in the presence of sortilin blocking antibodies. **Conclusion**: This is the first demonstration that functional sortilin is expressed by NK cells and can be activated by proNGF to mediate cell death.

POS-WED-197

HYPOTHALAMIC STUDY OF A NEW MODEL OF OBESE MICE

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Purpose: Diet-induced obesity is associated with hyperphagia and increased serum leptin, a central satiety regulator. The aim of these experiments was to study hypothalamic responses pertinent to appetite regulation in *foz/foz* mice, a murine model of Altröm syndrome – a form of monogenic obesity. Methods: Serum levels of leptin, neuropeptide Y (NPY) and agouti-related protein (AgRP) wa measured in 3, 8 and 18-week old *foz/foz* or wildtype (WT) mice (n≥5), fed with rodent chow or high-fat diet. Hypothalamic expression of the leptin receptor and the major neuropeptides involved in food intake regulation were measured by real time PCR, western blotting and immunofluorescent staining. Results: Serum leptin levels were similar in 3-week old *foz/foz* mice and WT littermates. By 8-weeks of age, serum leptin levels were increased in chow-fed *foz/foz*and high-fat fed WT mice, and were further elevated in high-fat fed *foz/foz*mice; levels continued to increase with time. Hypothalamic leptin receptor expression was increased in the hypothalamus of 8-week old high fat-fed vs chow-fed mice, irrespective of genotype; this difference was less apparent in older mice. Neither serum levels nor hypothalamic expression of NPY and AgRP appeared to be correlated with changes in serum leptin. Conclusion: The hyperleptinaemia induced by high-fat feeding is exacerbated in this genetic model of obesity, and is associated with higher hypothalamic leptin receptor expression, but without physiological changes in NPY and AgRP expression; these findings indicate altered central leptin regulation. Thus, the *foz/foz* model provides a new opportunity to study the central role of leptin in dysregulation of food intake.

POS-THU-198

EFFECTS OF SCHWANN CELL-DELIVERED NEUROTROPHIC FACTORS ON PERIPHERAL NERVE REGENERATION

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Purpose: After peripheral nerve (PN) injury it is sometimes necessary to use grafts to bridge the tissue defect and provide a substrate for regenerating axons. Use of PN autografts can be problematic, thus alternate sources of graft material must be found that promote survival of injured neurons and stimulate axonal regrowth. We quantified regeneration through acellular donor nerve sheaths reconstituted with Schwann cells (SCs) transduced ex-vivo with lentivirus (LV) encoding either brain-derived nerve factor (BDNF), ciliary neurotrophic factor (CNTF) or neurotrophin-3 (NT3). Control grafts were autografts, acellular grafts and sheaths containing non-transfected SCs. **Methods:** In anaesthetized (ketamine/xylazine) adult rats, grafts were inserted into 1cm gaps in the left peroneal nerve and tissue collected 10 weeks later (n ≥ 5 for each group). Axon counts and morphology in host and grafted PN were assessed in ultra and semi-thin cross sections, and in immunohistochemically stained longitudinal cryosections. **Results:** Within grafts, unlike intact nerves, axons were organized in discrete fascicles, especially obvious in LV-NT3 engineered nerves. Average axonal densities (βIIITubulin immunostaining) differed between graft types, the lowest number seen in LV-CNTF grafts (55 axons/mm; 89 axons/mm in intact PN). LV-BDNF and LV-NT3 grafts were thicker than intact nerves and the total number of axons in LV-NT3 grafts was higher (70 axons/section) than in any other graft type. In each group the number of axons in host PN proximal and distal to the grafts is under analysis. **Conclusion:** PN grafts containing SCs transduced with different neurotrophic factors develop different morphologies and differ in their ability to support axonal regeneration.

POS-WED-199

IDENTIFICATION OF NEURONS THAT EXPRESS GHRELIN RECEPTORS IN THE MEDULLA OBLONGATA AND SPINAL CORD

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The best-known effects of ghrelin are to cause growth hormone release and to increase feeding. However, ghrelin receptors (GHSR) are found in neurons in several brain regions and recent data indicate that GHSR agonists increase defecation and raise blood pressure by actions on neurons in the lumbo-sacral spinal cord. **Purpose**: The purpose of the study was to determine whether GHSR is expressed by neurons of autonomic control centres in the medulla oblongata and spinal cord. **Methods**: Fresh tissue was taken and extracted to detect GHSR mRNA by RT-PCR (n = 6). In addition, 12 rats were perfused with 4% formaldehyde and medulla and spinal cord were prepared for in situ localisation of GHSR mRNA. **Results**: GHSR and ghrelin mRNA were detected in extracts obtained from medulla oblongata and the rostro-caudal extent of the spinal cord, suggesting the presence of endogenous ghrelin signaling circuits in these parts of the CNS. The strongest GHSR in situ hybridization signals were obtained in the medulla, particularly in neurons of the nucleus ambiguus. GHSR expressing neurons were also found in the rostral and caudal ventro-lateral medulla, nucleus tractus solitarius, area postrema, ventral raphe and spinal trigeminal nucleus. GHSR-expression in the spinal cord was less conspicuous and was confined to small numbers of neurons in the intermedio-medial (IMM) column and intermedio-lateral (IML) nuclei, identified by nitric oxide synthase expression. **Conclusion**: Ghrelin receptors occur in neurons of several autonomic control centres. Despite the powerful effects of GHSR agonists on defecation centres in the lumbo-sacral spinal cord, only a small number of neurons in these centres express this receptor.

POS-THU-200

RESPIRATORY EFFECTS OF SOMATOSTATIN IN SUBNUCLEI OF THE VENTRAL RESPIRATORY COLUMN OF THE RAT: MECHANISMS OF RESPIRATORY SUPPRESSION

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Purpose: Somatostatin is expressed in the ventrolateral medulla (VLM) by neurons necessary for the genesis of breathing. Surprisingly little is known about the regulatory role of somatostatin in the VLM, other than that local administration induces apnoea. Thus, we examined the respiratory effects of microinjecting somatostatin into rhythmogenic (preBötzinger Complex), pattern forming (Bötzinger Complex) and spinally projecting (rVRG) respiratory subnuclei of the ventrolateral medulla in rat. **Methods:** Microinjections (20–50 nl) of SST (0.15, 0.45, 1.5 mM) were made into glutamate identified ventral respiratory subnuclei of urethane-anaesthetised (1.3g/kg ip), paralysed, vagotomised and artificially ventilated Sprague Dawley rats (n =42). Phrenic, vagus and splanchnic sympathetic nerves, end tidal CO2 and arterial pressure were recorded. **Results:** Bilateral microinjection into SST into Bötzinger Complex transformed eupneic phrenic burst patterns into prolonged inspiratory burst periods (apneusis), and abolished post-inspiratory activity of vagus and sympathetic nerves. SST in the preBötzinger Complex causes bradypnoea and induces sustained apnoea with higher doses. SST in the rVRG caused a depression of phrenic drive leading to apnoea. **Conclusion:** SST exerts powerful inhibition of respiratory neurons throughout the ventrolateral medulla and at all hierarchical stages of respiration: respiratory rhythm generation, pattern formation, bulbospinal and cranial nerve transmission. Identifying the natural stimuli that evoke somatostatin release is a priority.

POS-WED-201

MULTIPLE PROTECTIVE ACTIVITIES OF NEUROGLOBIN IN CULTURED NEURONAL CELLS EXPOSED TO HYPOXIA RE-OXYGENATION INJURY

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Purpose Oxidative stress is associated with the pathology of acute and chronic neurodegenerative disease. We have cloned a human neuroglobin (Nb) construct and over-expressed this protein in cultured human neuronal cells to assess whether Nb ameliorates the cellular response to experimental hypoxia-reoxygenation (H/R) injury. **Methods/Results** Parental cells transfected with a blank (pDEST40) vector responded to H/R injury with a significant decrease in cellular ATP at 5 and 24 h after insult. This was coupled with increases in the cytosolic Ca2+, and the transition metals iron (Fe), copper (Cu), and zinc (Zn) within the cell body as monitored simultaneously using x-ray fluorescence microprobe imaging. Parental cell viability decreased over the same time period with a ~4-5 fold increase in cell death (maximum ~25%) matched by an increase in caspase3/7 activation (peaking at a 15-fold increase after 24 h) and condensation of β-actin along axonal processes. Over-expression of Nb inhibited ATP loss and except for significant decreases in elemental sulfur (S), chlorine (Cl), potassium (K) and Ca2+, maintained cellular ion homeostasis after H/R insult. This resulted in increased cell viability, significantly diminished caspase activation and maintenance of the β-actin cytoskeletal structure and receptor-mediated endocytosis. **Conclusion** These data indicate that bolstering the cellular content of Nb inhibits neuronal cell dysfunction promoted by H/R insult through multiple protective actions including: (i) maintaining cellular bioenergetics; (ii) inhibiting Ca2+ influx; (iii) abolishing cellular uptake of Fe, Cu and Zn at the expense of S, Cl and K; and (iv) enhancing cell viability through inhibiting necrosis and apoptosis.

POS-THU-202

PRENATAL NICOTINE EXPOSURE IN THE FETAL BABOON INCREASES PARASYMAPATHETIC ACTIVITY AND RESULTS IN SEROTONERGIC AND NICOTINIC BRAINSTEM ABNORMALITIES: IMPLICATIONS FOR THE SUDDEN INFANT DEATH SYNDROME

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Purpose: The sudden infant death syndrome (SIDS) is postulated to result from sleep-related abnormalities in the brainstem's control of autonomic functions during a critical developmental period[1]. Maternal cigarette smoking during pregnancy, the major modifiable SIDS risk factor, increases risk 3-5-fold[2]. Previously, we reported serotonergic (5-HT) abnormalities in SIDS in regions of the medulla oblongata that modulate cardiorespiratory function[1], raising questions about 5-HT and nicotine (the major neurotoxic component in cigarette smoke) interactions in brainstem dysfunction. In a fetal baboon model we tested the hypothesis that prenatal nicotine exposure alters autonomic function in association with medullary 5-HT abnormalities. **Methods:** Instrumented pregnant baboons *(papio)* were infused with nicotine (0.5 mg/hr, i.v., n=5) or saline (n=5) from 87±1 to 160±2 days gestation (term=180d); human term equivalent. Fetal physiology recordings were collected from 129±1 days on and the fetal medulla (n=5 nicotine-exposed, n=6 controls) accessed for neuronal nicotinic receptor (nAChR) and 5-HT markers with immunocytochemistry and tissue autoradiography. **Results:** Prenatal nicotine exposure increased parasympathetic activity ($p=0.04$) which was associated with increased 5-HT_{1A} receptor expression ($p=0.04$) isolated to the raphé obscurus of the medullary 5-HT system, in conjunction with widespread nAChR abnormalities ($p<0.05$). **Conclusion:** Prenatal nicotine exposure may shift autonomic balance towards a parasympathetic prevalence due in part to abnormal 5-HT-nicotine interactions in the medullary raphé. [1]Kinney, H.C., et al., JNEN, 60, 228-47 (2001); [2]Mitchell, E.A., Med J Aust, 173, 175-6 (2000).

POS-WED-203

DOES THE EXTRACELLULAR CALCIUM SENSING RECEPTOR DETECT LUMINAL AMINO ACIDS IN THE INTESTINAL MUCOSA?

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Purpose Application of L-phenylalanine or L-tryptophan to the intestinal mucosa excites a reflex that triggers inhibitory junction potentials in the circular muscle. This involves release of serotonin (5-HT) from mucosal enterochromaffin (EC) cells, but how EC cells detect amino acids is unknown. One possible sensory receptor molecule is the extracellular calcium sensing receptor (CaSR), which is sensitive to aromatic L-amino acids. This study tested this possibility. **Methods** All experiments were performed on jejunum from guinea-pigs killed using procedures approved by the University of Melbourne Animal Experimentation Ethics Committee. Expression of CaSR mRNA in guinea-pig jejunal mucosa was tested using RT-PCR. Double staining fluorescence immunohistochemistry of cross-sections of fixed guinea-pig jejunum using antisera against the CaSR and against 5-HT (to label EC cells) was used to determine whether EC cells expressed CaSR protein. The sensitivity of the native receptor to different amino acids was examined using intracellular recording of reflexes in the circular muscle **Results** Guinea-pig mucosa was found to express CaSR mRNA. The guinea-pig transcript was 92% homologous with the human CaSR at the nucleotide level. Confocal analysis showed that all EC (5-HT) cells (n=180) also express the CaSR. Analysis of reflexes showed that native amino acid receptors are stereospecific: D-phenylalanine was ineffective when applied to locations from which L-phenylalanine evoked an inhibitory reflex (n=6). The receptor prefers aromatic amino acids: neither L-lysine nor L-leucine evoked reflexes at L-phenylalanine sensitive sites (n=4 each). **Conclusion** The ability of amino acids to evoke local inhibitory reflexes parallels their ability to activate the CaSR. CaSRs probably act as sensory transducers for luminal amino acids in guinea-pig jejunum.

POS-THU-204

SHAPES OF ENTERIC NEURONS REVEALED BY NEUROFILAMENT LOCALISATION DIFFER FROM THOSE REVEALED BY CYTOPLASMIC MARKERS

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Anti-neurofilament antibodies reveal the cytoskeletal architecture that influences neuronal shape, especially of large neurons. However, the largest neurons in guinea-pig enteric ganglia (type II neurons) were previously found negative for neurofilaments. Moreover, neuronal shapes revealed by neurofilament localisation in other species differ from the shapes of corresponding guinea-pig neurons. Purpose: The purpose of the study was to determine which neurons express high and medium weight neurofilaments (NF-H and NF-M) and the shapes that are revealed by neurofilament staining, in comparison to complete cytoplasmic staining. Methods: Wholemounts of guinea-pig myenteric and submucosal plexuses were prepared for immunohistochemistry and were stained with combinations of markers. Results: NF-H immunoreactivity occurred in all myenteric type II neurons (identified by IB4 binding), but these were never NF-M immunoreactive. On the other hand, 17% of neurons expressed NF-M. Many of these were uniaxonal neurons with spiny dendrites and nitric oxide synthase (NOS) immunoreactivity. NOS immunoreactivity revealed expansions of the cytoplasm that did not contain neurofilament immunoreactivity. Thus with NOS immunoreactivity they had the appearance of type I neurons. In the submucosa, type II neurons were NF-H immunoreactive, but the other neuron types, secretomotor and secretomotor-vasodilator neurons, were negative. Conclusion: We conclude that the apparent morphologies and the morphological classifications of enteric neurons are dependent on the methods used to reveal them. We conclude that spiny, type I, NOS immunoreactive, neurons are similar in human and guinea-pig, and that many of these are inhibitory motor neurons.

POS-WED-205

INFLAMMATION OF THE ILEUM HAS EFFECTS AT A REMOTE SITE, THE CELIAC GANGLION

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Inflammation of the small intestine causes changes in neuronal properties at the site of inflammation, changes in other gut regions and changes in dorsal root ganglion neurons. We have recently shown that neurons in the celiac ganglion are also affected, these neurons becoming hyper-excitable a week after an inflammatory stimulus (Dong et al, Neuroscience Letters, 444, 231-235, 2008). Purpose: This study was to determine whether changes in Na^+ channel expression and inflammatory cell infiltration are associated with the neuronal hyper-excitability that occurs in the celiac ganglion. Methods: Trinitrobenzene sulphonate was introduced into the lumen of the distal ileum of 12 anaesthetised guinea-pigs and the animals were allowed to recover for 7 days before tissue was taken. Celiac ganglia were extracted to determine mRNA levels of transcripts of target channels by real-time PCR. Other ganglia were processed for the localisation of neutrophils and T cells. Results: Inflammation of the ileum caused a significant increase in expression of the mRNAs of tetrodotoxin-sensitive Na^+ channels, 3.5 fold for NaV 1.2 and 1.5 fold for NaV 1.7, and a 2.5 fold increase in mRNA for the tetrodotoxin-resistant NaV 1.9 channel in the celiac ganglion. Neutrophils, normally rare or absent in the celiac ganglion, were increased in number, as were T-cells. Conclusion: Inflammation of the ileum causes immune-mediated changes in the celiac ganglia, although changes mediated through neural connections between the celiac ganglion and the ileum may also contribute. Neuronal hyper-excitability is associated with increased expression of voltage-dependent Na^+ channels.

POS-THU-206

HISTOLOGICAL CHANGES AND INFLAMMATORY CELL INVASION IN THE ILEUM AFTER A BRIEF INFLAMMATORY EPISODE

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The consequences of a brief gastroenteritis, notably changes in neural control of intestinal function, persist for long periods after obvious tissue changes have subsided. This appears to be due to the induction of long-term changes in neuronal excitability, but there is a lack of detailed information on the relation between tissue damage, inflammatory cell infiltration and neuronal changes. Purpose: The aim was to obtain a detailed description of the structural changes that occur after a single exposure to an inflammatory stimulus and to relate these to the appearance and proliferation of neutrophils and T cells in the tissue. Methods: Trinitrobenzene sulphonate was introduced into the lumen of the distal ileum of anaesthetised guinea-pigs and the animals were allowed to recover for from 3 hr to 56 days. The ileum at the site of hapten exposure and more proximal sites were taken for histology and histochemistry. Treated and sham animals (21 each) were compared. Results: At 3 hr the mucosal surface was denuded, by 6 hr there was substantial epithelial repair and this was complete by 1 day. Prominent phagocytic activity of epithelial cells occurred at 3 days. Damage was resolved at 7 days. Numbers of neutrophils rose quickly to 24 hr, subsided, but persisted to 56 days. T cells rose more slowly and subsided more quickly. Conclusion: In this model, overt tissue damage is over at a week, but low-level inflammation continues to 8 weeks, as do increases in neuronal excitability.

POS-WED-207

NEURONAL INJURY AND DEATH FOLLOWING INTESTINAL INFLAMMATION

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Damage to the neurons and their death following intestinal inflammation might lead to symptoms of pain and disorders of motility that persist long after the resolution of inflammation, the condition known as Irritable Bowel Syndrome (IBS). The current therapies of IBS are not effective and only symptomatic. We hypothesize that by preventing neuronal damage and neuronal death that occurs at the acute stage of inflammation we will target the triggering mechanisms of neuronal hyperexcitability, and, therefore, we can prevent the development of post-inflammatory IBS. **Purpose:** This study aims to investigate axonal damage and apoptosis of enteric neurons in guinea-pigs with trinitrobenzene sulfonate (TNBS)-induced ileitis. **Methods:** Inflammation was induced by injecting TNBS (30mg/kg in 30% ethanol) into the guinea-pig ileum. Segments of inflamed ileum were examined at 1 and 7 days after TNBS injection and compared to control ileum (total 24 guinea-pigs). Inflammation was quantified histologically and immunochemically. Nerve fibre bundles have been labeled with anti-βTubulin, GAP-43 and anti-phospho-Tau antibodies. The number of neurons in myenteric plexus has been revealed and quantified using anti-Hu antibody at 1, 7, 28 and 56 days after TNBS injection, followed by double-staining with anti-calbindin antibody specific to Dogiel type II neurons. Neurons undergoing apoptosis in myenteric plexus have been studied using in vitro fluorescent dye FLICA which labels activated caspases. **Results:** Inflammation caused an increase in number of processes projecting to the mucosa at day 7 after the TNBS injection (n=3). The processes were irregular and tangled. The number of nerve bundles was visualized and quantified in mid-villi sections (n=3). The total number of neurons decreased at day 1 and stayed the same at 7, 28 and 56 days after the induction of inflammation (n=3 at each time point). **Conclusion:** Intestinal inflammation causes damage to the neuronal processes in the mucosa and neuronal apoptosis.

POS-THU-208

MORPHOLOGICAL CHANGES IN SPECIFIC SUBTYPES OF MYENTERIC NEURONS FOLLOWING ISCHEMIA

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Damage following ischemia and reperfusion (I/R) is common in the intestine, and can be caused during abdominal surgery, in disease states and in intestinal transplantation. Most studies have concentrated on damage to the mucosa, although the observed post-ischemic consequences imply neuronal damage. Purpose: The study was designed to determine whether damage to neurons could be detected using morphological and immunohistochemical criteria, which could thus allow the time courses of changes and effects of protective agents to be evaluated. Methods: Mesenteric blood vessels of anaesthetised guinea-pigs were occluded for 0.5-1 hr and the animals were allowed to recover for 2 hr-28 days before tissue was taken. Observation in tissues from 24 sham and 24 I/R animals were compared. Results: Changes were observed in neurons with nitric oxide synthase (NOS) immunoreactivity (IR) and in type II neurons (NeuN-IR). The dendrites of the NOS neurons were distorted and swollen by two hours after I/R and remained enlarged up to 28 days. Total neuron profiles areas were increased by 25%, but the cell bodies were not changed significantly. Type II neurons were slightly reduced in cell size (only significant at 7 days) and exhibited some cell-surface distortion. Calretinin neurons were unaffected. Conclusion: There is a selectivity of effects of I/R injury on subclasses of enteric neuron. I/R causes specific changes in dendritic morphology of NOS neurons, which implies that there is a modification of the cytoskeleton. This may involve increased Ca^{2+} permeability, Ca^{2+}-dependent production of the NO free radical and cytoskeletal damage.

POS-WED-209

CALCIUM CHANNELS IN MYENTERIC NEURONS: MODULATION BY PREGABALIN AND GABAPENTIN

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Purpose: Calcium channels that mediate neurotransmitter release and contribute to the regulation of neuronal excitability are also linked to pain transmission following nerve injury or inflammation. Two compounds effective in the treatment of neuropathic pain, pregabalin and gabapentin, decrease calcium currents via binding the calcium channel auxiliary $\alpha_2\delta$ subunit. Here we investigated whether such compounds modulate calcium channel activity in myenteric AH neurons. **Methods:** Segments of ileum (adult guinea-pigs) were dissected into myenteric plexus and longitudinal muscle preparations for intracellular microelectrode recording and *in situ* hybridisation. **Results:** The effects of pregabalin (10-50μM) and gabapentin (50μM) upon AH neurons was evaluated by examining changes in the calcium hump on the falling phase of the action potential, and the calcium-dependent late after-hyperpolarising potential (AHP) (n=28). Both compounds failed to alter the action potential profile, but attenuated the late AHP, and reduced the enhanced calcium hump after it had been extended by TEA (10mM). Pregabalin administered following TEA and the N-type blocker ω-conotoxin GVIA (0.5μm), resulted in no further reduction in the hump. *In situ* hybridisation revealed mRNA for α_{1A} (P/Q-type) and α_{1B} (N-type) subunits in Dogiel type II neurons (the morphological correlate of AH). Conversely, whilst $\alpha_2\delta_1$ and $\alpha_2\delta_2$ subunits were prominent in myenteric ganglia, only faint labelling was observed in AH neurons. **Conclusion:** Pregabalin and gabapentin reduce calcium-mediated events in AH neurons, but not in a manner consistent with the block of N-type channels. *In situ* hybridisation confirms the presence of both P/Q-type and N-type channels in AH neurons, but suggest the auxiliary subunits targeted by pregabalin and gabapentin are not prominent in this population.

POS-THU-210

ALLEVIATION OF THE EFFECTS OF CHRONIC STRESS ON LONG-TERM POTENTIATION IN THE RAT HIPPOCAMPUS

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Purpose: Exposure to chronic stress has been shown to impair the function of the hippocampus. At least part of this impairment involves structural changes, including reduced apical dendrites and dendritic spines. These changes produce a decrease in long-term potentiation (LTP). A number of plant-derived odours have been shown to attenuate the effects of acute and chronic stress in different systems, including the sympathetic nervous system. In the present study, we examined the effect of Praescent™, a combination of stress-alleviating odours, on LTP in the rat hippocampus. **Methods:** Male Wistar rats (age 3 weeks) were subjected to chronic restraint stress for 4 hours each day/21 days, with concurrent exposure to Praescent™ or vehicle during the restraint period, or with no exposure to either odour. Control animals with or without exposure to each odour were also included. Following treatment, animals were anaesthetized with sodium pentobarbitone (60mg/kg i.p.). Field potentials from CA3-CA1 neurons were recorded using paired-pulse facilitation on in-vitro hippocampal slices from each animal. LTP was induced by applying high frequency (100Hz) stimulation. **Results:** We found that chronic restraint stress significantly reduced LTP ($P<0.0001$) in stressed only rats (n=5) compared to non-treated control rats (n=7). This effect was attenuated ($p<0.0001$) in rats stressed with exposure to Praescent™ (n=4), but not in rats stressed and exposed to vehicle alone (n=3). It was also observed that control rats exposed to Praescent™ (n=5) showed enhanced LTP compared to control rats without Praescent™ exposure ($P<0.0001$). **Conclusion:** Our results indicate that Praescent™ attenuates the effects of chronic stress on hippocampal neurons at the functional level.

POS-WED-211

PREVENTION OF STRESSED-INDUCED DEGENERATION IN CA3 HIPPOCAMPAL NEURONS BY PRAESCENT™

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Chronic stress induces many physiological changes such as dendritic atrophy and a decrease in spine densities within the CA3 and CA1 neurons of the hippocampus. Recently, it has been shown that plant-derived chemicals are able to reduce effects of chronic stress on the sympathetic nervous system and the endocrine system. **PURPOSE:** The aim of this study was to examine whether Praescent™ (plant-derived green odours) is able to attenuate the effects of chronic stress on the hippocampal neurons. **METHOD:** A morphological study was conducted to examine structural changes in the CA3 hippocampal neurons following chronic treatment of male Wistar rats. 3 week-old rats (n=12) were divided into four groups: controls, stressed only, stressed and exposed to Praescent™ and controls exposed to Praescent™ only. Rats were chronically stressed by restraint over 21 consecutive days for 4 hours. After 21 days, the rats were anaesthetized with Valabarb® and decapitated. The brains were removed and fixed in paraformaldehyde. The tissue was sliced using a vibratome before staining the slices with either Nissl or Golgi-Cox stains. Hippocampal morphology was analysed using a NeuroSterology equipped microscope. **RESULTS:** Dendritic atrophy and spine loss was evident following chronic stress. These effects are attenuated when rats were exposed to stress and Praescent™. **CONCLUSION:** This study indicates that some plant-derived odours may protect the hippocampus and other central nervous system structures from the damaging effects of prolonged stress exposure.

POS-THU-212

GAZE STRATEGY IN THE FREE FLYING ZEBRA FINCH (TAENIOPYGIA GUTTATA)

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Fast moving animals use spatial information derived from optic flow on their retina. Optic flow information from translational locomotion includes information about the three-dimensional composition of the environment and the heading direction of the individual, while optic flow experienced during a rotational self motion does not. Thus, a saccadic gaze strategy during locomotion segregating rotations from translational movement might be employed as an active behavioural strategy to facilitate extraction of spatial information from the visual input. It is known that the fly show saccadic head movement during flight. We analysed whether birds use such a gaze strategy by high-speed video recording zebra finches(n=10) during an obstacle avoidance task. The data show that in all flights the head is shifted in a saccadic fashion keeping it relatively straight between saccades. In addition, we could show that most tested birds(68%) prefer a two head saccade-strategy in obstacle-avoiding flight. These results indicate that birds use a gaze strategy that actively stabilizes their gaze during self movement. Thereby the acquisition of optic flow based three-dimensional information is simplified.

POS-WED-213

COLOUR DISCRIMINATION THRESHOLDS IN A TRIGGER FISH, RINECANTHUS ACULEATUS

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Purpose: Colours are used by animals in a wide variety of behaviours like food selection, mate choice and camouflage. However, the behavioural thresholds of colour discrimination have only been observed in a small number of species. We have used behavioural testing to obtain the colour discrimination thresholds of a trigger fish *Rinecanthus aculeatus*. **Methods:** Fish (n=3) were trained to discriminate between a reward colour stimulus and a series of non-reward test colour stimuli. The colours in the non-reward series were designed so that each successive colour was more similar to the reward one. **Results:** All fish achieved similar choice frequencies for the reward against the test colour series. A colour vision threshold was reached with the failure of the fish to discriminate colours similar to the reward. These results were compared with the predictions of the receptor noise limited colour opponent model (Vorobyev and Osorio, 1998, Proc R. Soc. London, 265:351-358). The obtained thresholds agree reasonably well with the assumption that noise in receptor channels is close to 3% of the signal. **Conclusions:** The *Rinecanthus aculeatus* conforms reasonably well with predictions made by the Vorobyev-Osorio model. These values can be compared to the psychophysically determined Weber fractions for human observer – about 2% for the long- and middle- wavelength cone mechanism and 8% for the short-wavelength cone mechanism.

POS-THU-214

PATTERN DISCRIMINATION IN REEF FISH

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Purpose: Many organisms analyse visual scenes in terms of signal strength at specific spatial frequencies and for many this represents a significant first step in visual processing. The outputs of this processing form a basis for higher level operations and visual discrimination tasks can often be solved on the basis of differences in spatial frequency alone. We have previously demonstrated that complex patterns on reef fish can be used for species and individual recognition. What these studies failed to clarify was whether the fish were using differences in spatial frequency, rather than differences in shape to perform this discrimination. **Methods:** Using behavioural experiments, fish were trained on one of two stimuli (clockwise and anticlockwise black and white swirls) and tested against the other pattern using a two-alternative choice approach. The trained stimulus had to be tapped at least twice before a food reward was given. Four damselfish (*Pomacentrus amboinensis*) were trained on each stimulus. The positions of the test stimuli were randomised across trials. **Results:** Within 15 sessions eight fish were able to discriminate between the two patterns reaching 75% choice frequency. On average, all fish identified the correct target in 80% [87.33, 70.82; upper, lower 95% C.I.] of choices. In both groups of fish, seven out of eight fish were able to discriminate between the two presented stimuli as their tapping distribution was significantly different from chance (Fischer's exact test $p<0.02$ in four cases and $p<0.04$ in two cases) **Conclusions:** *P. amboinensis* has the ability to learn to discriminate between two complicated black and white spiral patterns matched for spatial frequency content and varying only in direction of rotation.

POS-WED-215

NEURAL NETWORK MODEL FOR SEQUENCE LEARNING

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Purpose: Many tasks performed by animals, from navigation to speech, require learning and retrieving sequences of events. How such sequence learning can occur despite variation in the duration and timing of sequence elements is an open question. We propose a neural network structure, inspired by hippocampal neural correlates of navigation, that learns to recognize sequences composed of abstract symbols. **Methods:** The network consists of pools of leaky integrate and fire neurons, with one such pool for each symbol. When a symbol occurs it is represented by excitatory input to the corresponding pool, at a rate that is initially low, then increases, before ceasing. Periodic inhibition combines with the increasing input strength to result in sequence compression: during each cycle of the inhibition, neurons corresponding to recent symbols fire in the correct order. Synapses between pools corresponding to consecutive symbols are strengthened by spike-timing dependent plasticity and competitive heterosynaptic plasticity causes specialization of neurons to particular sequences. **Results:** Networks specified according to this scheme and trained on sets of overlapping and intersecting sequences correctly classify the sequences and are robust to variation in symbol duration. In a preset (i.e. nonplastic) network, signal-to-noise ratios range from 3.3 to 9.0 for symbol durations between 200 ms (n = 39) and 800 ms (n = 9) respectively. **Conclusion:** Sequence learning and recognition that is robust to variation in duration of sequence elements can be obtained through the mechanisms of sequence compression via periodic inhibition, spike-timing dependent plasticity, and heterosynaptic plasticity.

POS-THU-216

IDENTIFICATION OF NEURONS INVOLVED IN THE LEARNING OF FEAR MEMORY

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One of the fundamental questions in memory research is: Where in the brain does storage occur? Whereas particular brain regions have been have been strongly implicated in memory storage, the identification and location of the changes in the brain which underlie any memory are still unknown. **Purpose.** As a step to identify such changes, we aim to identify neurons which are specifically involved in learning and memory. **Methods.** We have developed a genetic approach, the fos-tau-lacz (FTL) mouse, to visualize and map functionally activated expression in neurons and their processes in the brain. We have used the FTL mouse to examine a classical model of learning, Pavlovian fear conditioning. **Results.** Using a single trial fear conditioning model, we have identified populations of neurons in several very defined areas of the brain which are specifically activated following learning (2 experiments, n=4 per experiment). One of these populations lies in a restricted part of the amygdala, a brain structure previously implicated in fear memory. We have begun to characterize these cells and our findings to date suggest they are predominantly excitatory (≥99%). **Conclusion.** We hypothesise that the neurons we have identified are directly involved in learning and formation of fear memories.

POS-WED-217

NERVE INJURY ALTERS REWARD-AVERSION BEHAVIOURS IN A SUBPOPULATION OF RATS

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Purpose: Neuropathic pain is characterised by sensory and affective changes. Sciatic nerve constriction injury (CCI) evokes hyperalgesia, allodynia and in ~30% of rats, changes in complex behaviours and endocrine function. Recent studies in humans and rats have shown alterations in the "reward-aversion" circuitry of the forebrain in neuropathic pain. These studies determined whether CCI altered "reward-aversion" behaviours. **Methods**: (1) Sucrose Preference: Eighteen, singly housed rats were provided with two drinking bottles for sixteen days, every second day one of these bottles contained 1% sucrose. After eight days of testing twelve of the rats were given a sciatic nerve CCI and the effects on sucrose intake determined. (2) Reward-Aversion: Eighteen rats, were placed in a two-chamber cage where preference for a "rewarding" food, or "aversion" of an unpleasant substance was evaluated behaviourally for twenty-nine days. CCI was performed on twelve rats and the effects on "reward" and "aversion" behaviours evaluated. **Results**: In uninjured rats water intake during the last eight days was identical (99%) to that during the initial eight days, in contrast sucrose intake increased by 120%. In CCI rats, water intake during the post-CCI period was unaffected (97% of pre-CCI period). However in half of the CCI rats (N=6) sucrose consumption decreased significantly to 75% of the pre-CCI levels. In four of the CCI rats reward and aversion behaviours decreased to <25% of the pre-injury scores. All CCI rats had sensory changes. **Conclusions**: These data suggest, similar to our earlier findings, and human clinical observations, that while CCI triggers sensory changes in all rats, there are distinct individual differences in the expression of complex behavioural changes to nerve injury.

POS-THU-218

REMOVAL OF STRIATAL PERINEURONAL NETS AFFECTS MORRIS WATER MAZE BEHAVIOUR IN MICE

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Purpose: Plasticity is a defining feature of the neostriatal circuit, yet much is unknown about its role in motor control and reward-mediated behaviour. We have recently shown the presence of perineuronal nets (PNNs), which have been shown to play significant roles in synaptic plasticity, to be expressed almost exclusively in the matrix compartment of the mouse neostriatum. PNNs have been shown to be associated with fast-spiking parvalbumin positive interneurons in cortical and subcortical structures. The identity of cells associated with PNNs in the caudate/putamen, and their importance to the functioning of striatal circuitry, however, has yet to be determined. **Methods:** In order to examine the identity of these PNN-encapsulated cells in the striatum, we are currently using double-fluorescence immunohistochemistry/ *Wisteria Floribunda* agglutin (WFA) binding to visualize PNNs and parvalbumin interneurons. To further investigate the role of PNNs in the striatum, we performed bilateral striatal injections of chondroitinase ABC (ChABC) in C57BL6/J mice and assessed their behaviour using the Morris water maze (n=4, saline control: n=4). **Results:** Double-fluorescence WFA binding/ parvalbumin immunohistochemistry shows that PNNs selectively surround parvalbumin-positive interneurons (n=3 animals). Our preliminary assessments show a trend towards differences in latency and swim path length between ChABC treated and untreated populations. **Conclusion:** PNNs are expressed on parvalbumin-positive GABAergic interneurons in the mouse neostriatum. Moreover, the selective removal of these proteoglycan structures may influence performance in a complex learning task.

POS-WED-219

LONG-TERM EFFECTS ON COGNITION OF CHRONIC RITALIN ADMINISTRATION TO NON-ADHD ADOLESCENT RATS

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Purpose: Children diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) are often chronically treated with psychostimulants, such as Ritalin, to normalise their dopamine systems. With increases in misdiagnosis of ADHD, children may be mistakenly exposed to chronic psychostimulant treatment. The aim of this study was to assess the effect of chronic Ritalin administration on cognitive development in non-ADHD and ADHD rats. **Method:** Male Wistar-Kyoto rats (WKY, non-ADHD) and Spontaneously Hypertensive rats (SHR, ADHD) were orally treated with either Ritalin (WKY-MPH, n = 12; SHR-MPH, n = 11) or distilled water (WKY-NO, n = 12; SHR-NO, n = 12) over 4 weeks throughout adolescence (PND 27 – 52). Ritalin was administered twice daily to model clinical dosing in children. Locomotor activity was measured during each week of treatment. After cessation of treatment, sensitivity to delay was measured using a delayed reinforcement (DRT) task followed by a radial arm maze (RAM) task to assess working memory. **Results:** Compared to WKY-NO, WKY-MPH showed significantly higher locomotor activity in the first week of treatment with no differences observed in weeks 2 – 4. This pattern was not reflected in SHRs, with SHR-MPH having a significantly higher locomotor activity than SHR-NO over the 4 weeks. On two occasions during the DRT, WKY-MPH chose the delayed reinforcer significantly less than WKY-NO. No treatment differences were found in the SHRs during the DRT. No treatment differences were found in the RAM. **Conclusion:** These results show different motor stimulant effects with repeated administration of oral Ritalin between the two rat strains. The DRT data suggest that cognitive deficits remain in adult non-ADHD (misdiagnosed) animals chronically treated with Ritalin throughout adolescence.

POS-THU-220

THE IMPACT OF CHRONIC RESTRAINT STRESS ON REGULATING BEHAVIOURAL AND GLIAL CHANGES IN THE ADULT RAT

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Purpose: Chronic unpredictable stress has been implicated in the development of a variety of affective disorders including depression and anxiety. Recent observations suggest that glia may play a crucial functional role in regulating these behavioural changes and its associated neural correlates following chronic stress. The current study has three aims (1) to confirm the effectiveness of restraint as a chronic stressor, (2) to assess the changes in the density of a number of glial markers following chronic restraint stress and (3) to observe the impact of stress on behaviour. **Method:** Male SD rats (n=30) were randomly allocated to three experimental conditions over a 14 day protocol: 1 x 1 hr restraint/day, 2 x ½ hr restraint/day, or 1 minute handling/day. Following stress, animals were subjected to sucrose preference testing. **Results:** The results indicated a significant reduction in weight gain for both restraint groups (all *p*'s<.01) during the stress period. Animals in the restraint group showed a significant decline in the duration of struggle over the first fifteen minutes of restraint ($p<.05$). The total duration of struggle was significantly less on subsequent restraint days ($p<.05$). Furthermore, we observed a significant decrease in sucrose preference for both restraint conditions (all *p*'s<.05) for one week following stress. **Conclusion:** The results demonstrated the efficaciousness of the chronic restraint model at inducing depressive-like behaviour, as indicated by decreased sucrose preference. In the context of the observed anhedonia, the reduction in struggle may be suggestive of learned helplessness. We are now in the initial stages of evaluating the effect of restraint on the aforementioned glial markers. The information derived from the current study will contribute to our understanding of how chronic stress alters neural architecture and in turn how such alterations influence emotional regulation.

POS-WED-221

THIAMINE DEFICIENCY IN RATS PRODUCES MEMORY DEFICITS ON SPATIAL TASKS THAT CORRELATE WITH THALAMIC SEROTONERGIC PARAMETERS

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PURPOSE: In previous studies, our group and other authors showed that experimental animal models of Wernicke's syndrome, neurodegenerative disease caused by deficiency of thiamine (DT), generates irreversible damage to spatial learning and memory. Differences in the performance of animals in cognitive tasks that involve aversive stimuli can be influenced by issues related to emotional components, which in turn could be associated with dysfunction in the serotonergic system. One of the aims of this study was to determinet whether the effects of DT on the animal performance in the Morris Water Maze (LAM), in which the water is an aversive stimulus, would be correlated with changes in the central serotonergic system. METHODS:We used male Wistar rats, three months of age, divided into two groups of n=16, whether or not subjected to an episode of severe DT. The concentrations of serotonin (5-HT) and the acid metabolite 5-hydroxy-indolacético (5-HIAA) were measured in the thalamus, a region mostly affected by the DT, using the high-performance liquid chromatography. RESULTS: Statistical analysis showed a significant effect ($p < 0.05$) of the deficiency of thiamine in the acquisition of the spatial task. However the animals in both groups were able to learn the task. We observed that the animals had disabled a significant increase in [5-HIAA] ng /g and in the rate of renewal ([5-HIAA] / [5-HT]) of the serotonergic system in the thalamus. Also observed a significant positive correlation between the [5-HIAA] and performance of animals in training session of LAM. CONCLUSIONS: We conclude that a serotonergic dysfunction in the thalamus, caused by a DT, may lead to impairment in the acquisition of a spatial learning task.

POS-THU-222

STIMULUS PATTERN DEPENDENCE OF THE ALZHEIMER'S DISEASE Aβ42 PEPTIDE'S INHIBITION OF LONG TERM POTENTIATION IN MOUSE HIPPOCAMPAL SLICES

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Activity dependent synaptic plasticity, and particularly long term potentiation (LTP) of synaptic strength in the hippocampus, is considered to represent a major cellular mechanism by which learning and memory is expressed in brain. Increasing evidence has pointed to inhibition of LTP by soluble Aβ42 oligomers as central in the etiology of the learning and memory deficits that are hallmarks of Alzheimer's Disease. These effects are thought to occur by an interaction between Aβ42 and certain cellular effectors that induce LTP, however, the precise identity of the Aβ42-interactive signaling molecules is unknown. Identification of such effectors is made more difficult because LTP induced by different stimulation protocols is often expressed through heterogeneous signaling pathways. The aim of this study was to compare two stimulation protocols, high frequency stimulation (HFS) and theta burst stimulation (TBS), using mouse hippocampal brain slices treated with Aβ42 peptide. Results show that untreated control brain slices, tetanized with either HFS or TBS, gave similar levels of LTP and post tetanic potentiation (PTP) suggesting that the response induced by either protocol was comparable. Aβ42 peptide significantly blocked LTP and PTP induced by HFS and not by TBS stimulation protocols. Specific NMDA receptor antagonists D-AP5 and ifenprodil, both blocked LTPs induced by HFS or TBS. We propose that certain signaling effectors other than the NMDA receptor, which are differentially involved in the induction of LTP by TBS and HFS, may be responsible for this resistance to Aβ42 dependent inhibition of TBS-induced LTP.

POS-WED-223

NEURAL ACTIVITY IN THE AMYGDALA DURING SEXUAL BEHAVIOUR

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Purpose: The amygdala is highlighted as a critical structure in normal sexual function, seen extensively in animal and human research. Recent functional imaging research suggests a contrasting role of the amygdala during different phases of sexual behaviour, with increases in neural activation seen during sexual arousal, and decreases in neural activation during genital stimulation and ejaculation/orgasm. This study investigated multi-unit activity in the amygdala during sexual behaviour in rats to examine patterns of neural activity across stereotyped sexual behaviour. **Methods:** Animals (n=5) were implanted with 16 channel electrodes into the right amygdala. Animals were subjected to a series of sexual behaviour tests (n=12) for 30 minutes during their active period. Continuous neural data was acquired using a wireless recording system that enabled animals to interact normally. Behaviours recorded included anogenital investigation, general body investigation, mount, mount with intromission, and mount with ejaculation. **Results:** All animals displayed sexual behaviour, however no animal reached ejaculation. Results revealed a significant anticipatory rise in neural activity from baseline values preceding each behaviour, a drop back to baseline levels at behaviour onset, and a significant sustained rise in neural activity during each behaviour. **Conclusion:** The anticipatory rise in neural activity suggests a role in processing sexually salient cues preceding behaviour onset. The drop in activation at behaviour onset before a sustained rise during behaviours suggests sexual arousal and behaviour may be processed as separate events. Also, sustained deactivation may only been seen with ejaculation, as previous research suggests neural deactivation releases inhibitory control of the amygdala to enable orgasm/euphoric events. Further research is necessary to support the results.

POS-THU-224

ASYMMETRY IN HIPPOCAMPAL DENTATE GYRUS MORPHOLOGY PREDICTS DISABILITY FOLLOWING PERIPHERAL NERVE INJURY

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Purpose: Animals respond to stress, threat or pain with either a proactive or reactive coping response. We have shown that Sprague-Dawley rats can be characterised into three distinct groups based on assessment of coping style across a number of stressors: (i) Proactive, (ii) Reactive and (iii) Shifters. Shifters are those rats, which fail to use either a proactive or reactive coping response consistently. Chronic constriction injury (CCI) of the sciatic nerve produces pain in all rats, but disabilities in only a subpopulation (~30%). We have shown that it is the "shifters" that develop pain and disability (i.e., fail to cope) following CCI. Hippocampal asymmetries have been correlated with failure to cope in a number of stress paradigms, these experiments aimed to answer: 1) do rats with different coping styles have differing hippocampal morphology?; 2) does CCI change hippocampal morphology?; and 3) do "shifters" and rats with pain and disability following CCI have similar hippocampal morphology? **Methods**: The volume, length and symmetry of the dentate gyrus (DG) was determined histologically from serial coronal brain sections for rats characterised for preferred coping style (n=22), and for rats following CCI (n=12). **Results**: Distinct coping styles are reflected in significant differences in hippocampal morphology. The DG is significantly longer and greater in volume in proactive vs. reactive rats. CCI evokes a significant reduction in the volume and length of the DG in all rats. Significant asymmetry between left and right hippocampal volumes were found in "shifters" and rats with pain and disability following CCI. **Conclusions**: Pre-injury asymmetry in the volume of the DG is a predictor of the development disability following CCI.

POS-WED-225

A SINGLE EXPOSURE TO AN ENRICHED ENVIRONMENT STIMULATES THE ACTIVATION OF DISCRETE NEURONAL POPULATIONS IN THE BRAIN OF THE *FOS-TAU-LACZ* MOUSE

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Storage of experience, including learning and memory, is thought to involve plasticity within pre-existing brain circuits. One model for looking at experience-dependent changes is environmental enrichment (EE), which involves exposing animals to a complex novel environment. Animals exposed to EE have previously been shown to exhibit a variety of behavioural and structural alterations in the brain, including decreased stress, improved learning and memory, altered levels of immediate early genes and synaptic change in the visual cortex. **Purpose:** We were interested in understanding what regions of the brain are activated during the initial stages of EE. **Methods:** We used the *fos-tau-lacZ* (FTL) transgenic mouse to examine changes in functional activation throughout the brain after a single exposure to EE. Female C57BL6 FTL mice (n=14) were divided into two groups; enriched and home cage. **Results:** We found that early in the process of EE, there was a high level of FTL expression in a series of brain regions in the enriched group compared to the home cage group ($p<0.001$), indicating that multiple circuits were activated. These regions include the claustrum, infralimbic cortex, hippocampus, amygdala and the hypothalamus. **Conclusion:** We believe that EE stimulates an initial strong increase in activation of multiple functional circuits. These circuits are presumably involved in the initial response of the animal to the enriched environment.

POS-THU-226

EXPERIENCES DURING PARALYSIS: VISION AND HUMOUR

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Purpose: Interventions in awake paralysed volunteers tested a central, intentional component to one's internal visual model and the importance of physical accompaniments to subjective emotional experience. Methods: Human volunteers (n=5) were pharmacologically paralysed for an encephalographic study, including tasks of attempted eye movement and humour. The study was approved by the Flinders Clinical Research Ethics Committee. The subjects reclined in a supported chair and were ventilated after paralysis (cisatracurium, 20 mg iv). In illumination, subjects were requested to focus alternately on the faces of investigators standing on the left and the right within peripheral vision. In darkness, subjects were instructed to look away from a point source of light. Subjects were then told the same joke. Subjects were to report experiences after reversal of paralysis. Results: With attempted eye movement in illumination, one subject had an illusion of environmental movement and the remaining four experienced detailed vision in their lateral fields. In darkness, four subjects reported movement of the target light in the direction of attempted eye movements. With the joke, four subjects found the mirth experience less enjoyable than expected and, in three subjects, even the intrinsic merit of joke was perceived to be less funny at the time than when it was recounted subsequently. Conclusions: Internal visual models receive intended ocular movement information directly from oculomotor centres. The perceived merit of a joke and the full enjoyment of humour appear to require awareness of its physical correlates

POS-WED-227

METHAMPHETAMINE, ETHANOL AND THEIR COMBINATION: SHORT-TERM EFFECTS ON MEASURES OF ANXIETY AND MEMORY IN THE RAT AFTER BINGE TREATMENT

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Purpose: Methamphetamine (MA) is a powerful psychomotor stimulant that is often used in conjunction with Ethanol (EtOH), however the neurobiology of this combination is poorly understood. The aim of the present study was to measure the effects on anxiety and memory of 'binge' pattern exposure to MA or EtOH alone or in combination. **Methods:** Young adult male rats (n =49) received four "binge" treatments which occurred weekly for 4 weeks and consisted of the following: 2 injections, one each hour on two consecutive week days, each injection comprised of MA (5mg/kg), EtOH (1g/kg {30% v/v}), MA (5mg/kg) + EtOH (1 g/kg) or MA (2.5mg/kg) + EtOH (1g/kg {15% v/v}) or vehicle. Drugs were administered at hot ambient temperatures, 28°C, to simulate nightclub conditions. One week following the four weeks of "binge" treatments social interaction, anxiety-related behaviour on the elevated plus maze (EPM) and novel object recognition (NOR) were assessed. Spatial reference and working memory on the Morris water maze were assessed on the following 19 days. **Results:** The high and low MA/EtOH treated group displayed a trend towards heightened anxiety on the EPM. The high MA/EtOH group engaged in significantly more adjacent lying and low MA/EtOH showed significantly more rearing in social interaction. All treatment groups spent less time exploring objects in NOR when compared to vehicle treated animals, however there were no differences on any other recognition, reference or working memory tasks. **Conclusion:** These results suggest that combined MA/EtOH may lead to different behavioural changes when compared to the use of either drug used alone, notably in social behaviours.

POS-THU-228

THE AFFECTS OF CALORIE RESTRICTION ON THE ACUTE IMMUNE RESPONSE

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Purpose: Calorie restriction (CR) has been shown to have health promoting benefits. It alters the release of some cytokines and reduces mortality after exposure to a bacterial infection. Although cytokine responses after CR have been investigated sickness behaviour (fever, anorexia, and cachexia) has not. The purpose of the present study was to examine the effect of CR on the development of sickness behaviour. **Method:** Adult male C57BL/6J mice were exposed to either a 25% restriction (CR25%) or 50% restriction (CR50%) for 28 days. On the 29th day the mice were injected IP with 50µg/kg of lipopolysaccharide (LPS). Changes in body temperature, locomotor activity, body weight, and food and water intake were determined. **Results:** CR50% mice demonstrated a full attenuation of sickness behaviour in comparison to controls; however, CR25% mice also showed a partial attenuation of sickness behaviour. CR25% mice displayed a shorter-lived fever with the same peak compared to the controls ($p <.001$), whereas the CR50% mice did not develop fevers ($p<.05$ to $p <.001$ for the duration of the fever in controls). There were two distinct groups of CR25% mice, those with fevers (n=7), which still reached the same peak as controls but with a shorter duration, and those without (n=3) ($p <.001$). Both CR25% and CR50% mice showed no sign of anorexia ($p <.001$) and reduced cachexia (at $p <.001$). **Conclusions:** CR results in a suppression of sickness behaviour in a dose dependent manner, which may be due to CR causing a reduction in metabolism and/or influencing several central nervous, endocrine and immune mechanisms.

POS-WED-229

SEROTONIN 5-HT4 RECEPTORS IN THE RAT NUCLEUS ACCUMBENS ARE INVOLVED IN MDMA-INDUCED APPETITE SUPPRESSION

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Purpose: It is recognised that serotonin (5-HT) release is a predominant mechanism of 3,4-methylenedioxymethamphetamine (MDMA) in the brain, yet characterisation of the specific receptors involved in the behavioural effects of MDMA, such as appetite suppression, is ongoing. The appetite suppressant effects of MDMA are attenuated in 5-HT4 knockout mice (Jean et al., 2007, PNAS 104:16335-40), however the location of these receptors involved in these effects are unknown. A key brain region involved in feeding and reward processes is the nucleus accumbens (NAc). 5-HT4 receptors are located in the NAc and we aimed to investigate the role of 5-HT4 receptors in this brain region in the appetite suppressing effects of MDMA. Methods: Male Hooded Wistar rats underwent surgery for the implantation of bilateral NAc microinjection cannulae (26 Ga) under isofluorane anaesthesia. Following 5-7 days of recovery the rats received bilateral microinjections of the 5-HT4 antagonist RS39604 (0, 0.3, 1 or 3nmol) into the NAc immediately prior to either saline (1 ml/kg, i.p.) or MDMA (10 mg/kg, i.p.) administration. Food and water intake was then measured for 3 hours (n=24). In a separate group the effect of RS39604 on MDMA- or saline-induced locomotor activity was measured for 3 hours (n=12). Results: Our results revealed that RS39604 (1nmol) significantly increased food intake after 1 hour in MDMA treated rats but not saline treated rats. Measures of weight change, water intake or locomotor activity were not altered by antagonist administration. Conclusions: These results demonstrate that 5-HT4 receptors in the NAc are involved in the appetite suppressant effects of MDMA, but do not mediate MDMA-induced water intake or locomotor activity.

POS-THU-230

EARLY DIETARY INTERVENTION AND HIGH FAT DIET INDUCED MATERNAL OBESITY INFLUENCES OFFSPRING CENTRAL APPETITE REGULATORS

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Purpose: The maternal nutritional state influences the development of offspring hypothalamic appetite regulators. We examined the relationship between the degree of maternal overfeeding and central appetite regulators in offspring. **Method:** We generated three levels of obese F1 female rats from chow and high fat diet (HFD) fed F0 founders. F1 females from chow fed F0 were raised in normal (CN) or small (CS) litters and fed chow; a third group from HFD fed F0 was raised in normal litters and fed HFD (HN). F1 females generated F2 male offspring who were fed either chow or HFD, yielding 6 groups: CN-chow, CN-HFD, CS-chow, CS-HFD, HN-chow and HN-HFD (n=12-17). **Results:** Compared to CN offspring, those of HN dams consuming chow and HFD were significantly heavier with elevated food intake and 100% and 62% increases in fat mass respectively (all $P<0.01$); offspring from CS dams were significantly heavier with 50% increases in fat mass ($P<0.01$) only in chow diet group. Importantly, HN offspring showed elevated leptin levels compared to CN offspring regardless of their diet ($P<0.01$). Hypothalamic orexigenic neuropeptide NPY and anorexigenic neuropeptide POMC mRNA were not changed by maternal overnutrition. Interestingly hypothalamic leptin receptor activator STAT3 and suppressor SOCS3 mRNA expression were significantly elevated in HN offspring consuming HFD ($P<0.05$, n=8). **Conclusion:** Our data suggest that in F1 dams can program adiposity to F2 offspring, with a greater impact of HFD induced maternal obesity. Upregulation of hypothalamic SOCS3 mRNA expression suggests central leptin resistance due to maternal HFD and F2 offspring HFD consumption. Additive effects were observed when F2 animals consumed HFD.

POS-WED-231

SPECIFIC GENE EXPRESSION IN THE RAT PERIAQUEDUCTAL GREY PREDICTS THE ANIMAL'S PREFERENCE FOR PROACTIVE, REACTIVE OR SHIFTING COPING STYLES

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Purpose: Chronic neuropathic pain is characterised by both sensory and affective changes. We have shown that following sciatic nerve constriction injury (CCI), 30% of rats develop a persistent disability in their complex behaviours and endocrine function. Rats that fail to adopt a consistent coping style (i.e., either proactive or reactive) to physical and/or psychological stressors, are most vulnerable to the development of disabilities after injury. The periaqueductal grey (PAG) is a brain region critical for the expression of emotional coping behaviours. In these experiments we aimed to determine whether rats with proactive, reactive or "shifting" coping styles were characterised by specific patterns of gene expression in the PAG. **Methods**: Rats (N=32) were characterised as either, proactive, reactive or shifters using 25 behavioural criteria, in a well characterised behavioural test battery. 24 genes of interest were identified as having significantly different expression between behavioural groups using Affymetrix GeneChips. RT-PCR was used to determine gene expression levels in the PAG following behavioural testing, compared to a naive control population of rats (N=64). **Results**: Significantly higher expression levels of Cd200, CCK and Pde1b and significantly lower levels of Mapk2, Mapk8, and TH characterised proactive rats, whereas significantly lower levels of BAX:Bcl2, CCK and TpH2 characterised reactive rats. Rats with a shifting coping style did not differ significantly from the control population for genes known to be regulated selectively by CCI: Bax/Bcl2, Camk2b, CCK, Cnr1, GFAP. **Conclusion**: Taken together with the earlier observation that "shifter" rats show greatest vulnerability to developing disabilities, the degree of gene regulation we have identified after CCI is more dramatic than first appreciated.

POS-THU-232

MOLECULAR FUNCTION OF GTF2IRD1, A TRANSCRIPTION FACTOR IMPLICATED IN WILLIAMS-BEUREN SYNDROME

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Purpose: GTF2IRD1 is a member of the TFII-I family of transcription factors that is hemizygously deleted in Williams-Beuren syndrome (WBS). The neuropathological features of WBS include impairment in visuospatial processing and mild mental retardation, but hypersociability is the most readily observed characteristic. We generated a *Gtf2ird1* knock-out (KO)-*lacZ* knock-in mouse which revealed that *Gtf2ird1* is expressed in the inhibitory GABAergic neurons. The *Gtf2ird1* KO mouse was also characterised by reduced anxiety, altered sociability and reduced susceptibilty to pentylenetetrazol-induced seizures. Taken together, these data implicate a potential role for *Gtf2ird1* in GABAergic functions. The function of *Gtf2ird1* at the molecular level is still largely unknown. Our aim is to identify downstream gene targets of *Gtf2ird1* in the brain. **Methods:** Microarray analyses were done to compare gene expression profiles of wild-type and KO mice. Pooled RNA samples were obtained from three specific regions of the brain: cerebral cortex, hippocampus and olfactory bulb (n=7) in each group of mice (n=2). **Results:** Pairwise comparison within each brain region revealed a total of 32 genes that are deregulated by at least 2-fold in the KO mice. Common to all three brain regions were genes involved in chromatin regulation which were down-regulated 2.5-fold in the KO mice. Other hormone-related genes were up-regulated by 2- to 3-fold in the hippocampi of the KO mice. qRT-PCR and Western blotting experiments are underway to confirm these microarray data. **Conclusion:** Our gene profiling analyses on *Gtf2ird1* KO mice identified interesting candidate genes that are downstream of its transcriptional activity. In particular, identification of genes implicated in chromatin remodelling suggests that GTF2IRD1 may play a role in the epigenetics of WBS.

POS-WED-233

INSULIN-LIKE PEPTIDE-3 RECEPTOR LGR8 IN MOUSE BRAIN: ENRICHMENT IN SENSORIMOTOR/LIMBIC CIRCUITS AND BEHAVIOURAL EFFECTS OF ICV INSL3

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Background: Leucine-rich repeat-containing G-protein-coupled receptor 8 (LGR8) is the native receptor for the peptide/hormone, insulin-like peptide 3 (INSL3). We recently reported the distribution of LGR8 in rat brain [Neuroscience 156 (2008) 319-333], where it is abundantly synthesized in midline thalamic neurons and expressed on their soma and axons/terminals in a range of target areas, including striatum and cortex. Activation of striatal LGR8 by INSL3 produced stereotypic sniffing in rats. **Purpose**: On the basis of a comparative mapping that revealed important differences in the LGR8 distribution in mouse brain, this study examined the effect of central LGR8 activation on sensorimotor behaviour in mice. **Methods**: Mice were stereotaxically implanted with indwelling guide-cannulae aimed at the lateral cerebral ventricle, allowed to recover for 7 days, and subjected to daily handling prior to experimentation. After infusions of INSL3 (10-80 pmol) or vehicle icv, mice were assessed in the home cage, automated locomotor cell, large open-field (LOF) and in an acoustic-startle assay. **Results**: INSL3 (10-80 pmol) produced a dose- and time-related increase in grooming behaviour in the home cage (max 2-fold above vehicle at 5-15 min, n = 6-8 per treatment, ANOVA $p<0.01$). INSL3 (40 pmol, n = 4-5) had no effect on locomotor activity in the LOF or locomotor cell, over 10/60 min, respectively. Effects on INSL3 (40 pmol) on prepulse inhibition of ASR were variable. **Conclusion**: Our anatomical and behavioural findings suggest a role for LGR8 in sensorimotor control in the mouse, via presynaptic regulation of glutamate transmission in cortical-basal ganglia circuits.

POS-THU-234

ANXIOLYTIC AND ANTIPSYCHOTIC EFFECTS OF THE NON-PSYCHOACTIVE PLANT CANNABINOID CANNABIDIOL – BEHAVIOURAL AND C-FOS IMMUNOHISTOCHEMICAL EVIDENCE

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Purpose: The non-psychoactive phytocannabinoid cannabidiol (CBD) may have anxiolytic and antipsychotic potential. Using the well-characterised effects of the psychoactive cannabis constituent Δ^9-tetrahydrocannabinol (THC) for comparison, we investigated the acute and chronic behavioural profile of CBD in a battery of schizophrenia-relevant tests in adult male C57BL/6 mice and examined its effects on dexamphetamine-induced neuronal activity using c-fos immunohistochemistry. **Methods:** Mice received 21 daily injections of vehicle, THC (0.3 - 10 mg/kg) or CBD (1 - 50 mg/kg). Behavioural testing was performed on day 1 (open field and prepulse inhibition) and days 15 – 21 (light-dark test, elevated plus maze, social interaction, prepulse inhibition and open field). On day 21, 45 min after the final cannabinoid injection, mice were injected with vehicle or dexamphetamine (5 mg/kg), placed in an open field for 45 min then perfused transcardially with saline and paraformaldehyde (4%). Immunohistochemical analysis for c-fos protein expression in frontal brain regions was performed. **Results:** THC produced the classical CB_1 receptor-mediated behavioural 'tetrad' of hypolocomotion, analgesia, sedation and hypothermia, while CBD had no effect on these measures. CBD (50 mg/kg) was anxiolytic in the open field and light-dark tests compared to the anxiogenic effect of high dose THC (10 mg/kg). Acutely, THC (0.3 and 10 mg/kg) and CBD (5 and 50 mg/kg) increased prepulse inhibition; this effect was retained during chronic treatment in mice treated with CBD (1 mg/kg). Importantly, CBD (50 mg/kg) reduced dexamphetamine (5 mg/kg)-induced hyperlocomotion. Preliminary immunohistochemical analysis suggests that pretreatment with CBD (1 and 50 mg/kg) alters the profile of c-fos immunoreactivity produced by dexamphetamine in the nucleus accumbens and prefrontal cortex. **Conclusion:** These results suggest an antipsychotic-like action of CBD. Further work will investigate the effect of acute CBD on schizophrenia-relevant behavioural measures in the heterozygous neuregulin 1 transmembrane domain mouse, a genetic mouse model of schizophrenia.

POS-WED-235

HEART RATE VARIABILITY AND QT INTERVAL VARIABILITY ARE NOT CORRELATED WITH CARDIAC NORADRENALINE SPILLOVER IN PATIENTS WITH DEPRESSION AND PANIC DISORDER

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Purpose: Various indices of heart rate variability (HRV) are used for the assessment of cardiac autonomic influences. It is however rarely acknowledged that these indices reflect exclusively neural outflow to the pacemaker region, and the relationship between HRV and autonomic effects on the ventricular myocardium are unknown. Attempts were made to employ for this purpose indices derived from the ECG QT interval that is linked to the ventricular repolarization. To address these issues in the most direct way, we computed HRV and QT variability indices and correlated them with the amount of noradrenaline spillover from the heart. **Methods:** The study was performed in 17 subjects (12 with major depression disorder and 5 with panic disorder). Cardiac noradrenaline spillover was assessed with direct catheter technique coupled with norepinephrine isotope dilution methodology. We compured standard HRV measures in the time and frequency domain (meanNN, SDNN, RMSSD, VLF, LF, HF, LF/HF ratio), and short-term heart rate complexity was quantified using detrended fluctuation analysis, symbolic dynamics and sample entropy. QT variability was quantified using QT variability index, QT variance, rate-corrected QT, QT/RR ratio, hysteresis of QT rate adaptation, global regression residual of the [QTi, RRi] fit, and QT/RR coherence. **Results:** Overall, none of HRV indices, short-term heart rate complexity indices, or QT interval variability indices correlated with cardiac noradrenaline spillover. **Conclusions:** Most currently available ECG-derived indices do not reflect sympathetic influences in the myocardium.

POS-THU-236

RETINOTOPIC ORGANIZATION OF ANTI-FACE AFTEREFFECT

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Purpose: One of the challenges for the visual system is to piece together the samples from successive fixations to construct the stable representation of the world that we all consciously perceive. The purpose of this study was to demonstrate if anti-face aftereffect transfers across saccades at all, whether in the same spatial position or not. **Methods:** Six subjects participated in four test conditions. In baseline condition, observers fixated centrally and were presented with test stimulus in one of two peripheral locations. In remaining conditions, the trial began with a 5s adaptation period, followed by a blank, a 250ms test stimulus. In full-adaptation condition, both adaptor and test were presented in same peripheral location. We then re-measured the effects, with a saccade intervening between adaptor and test, when the two stimuli were in the same spatial position (spatiotopic) and when in different spatial positions (matched in eccentricity and retinotopic displacement). **Results:** Anti-face adaptation did not transfer across saccades, consistent with earlier studies that used simpler stimuli with the same method. **Conclusion**: Our result is in contrast with some of the previous studies, which demonstrated that the basic shape information is combined across saccades. We propose those spatial aftereffects found in some studies, could be originated from a general, small remote aftereffect enhanced specifically at the spatiotopic locus, probably by specific attention toward that point.

POS-WED-237

WHY TERTIARY EDUCATION IS BAD FOR YOUR EYES!

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Purpose: Myopia is the most prevalent refractive error of the eye, and has been attributed to a range of genetic and environmental factors, including stress. The present investigation examined whether an acute psychological stressor (assessable oral presentation) contributes to the myopic shift previously reported to occur in University students over a 3 year degree. Method: Study One explored the association between refractive state and demographic characteristics of first (n = 111) and senior year (n = 64) Australian University students. Study Two, compared sympathetic nervous system activation (indicated by heart rate, salivary cortisol, systolic and diastolic blood pressure), intraocular pressure (IOP) and refractive state in first year students (n = 44) at baseline and following an acute stress condition. Results: Study One; Myopia was significantly greater in the senior cohort and was associated with greater amounts of near work. Study 2; Levels of myopia significantly increased following the acute stress condition, in conjunction with elevated cortisol levels and a non-significant increase in IOP. Conclusion; Acute stressful events at University contribute to a myopic shift in refraction over the course of tertiary study, though whether these shifts are transient or permanent is as yet unknown.

POS-THU-238

CHANGES IN FUNCTION AND GENE EXPRESSION IN CENTRAL NUCLEI AFTER COCHLEAR LESIONS

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Changes in auditory input as a result of cochlear damage can trigger a range of changes in central nuclei of the ascending auditory system, including spontaneous hyperactivity and map plasticity. The changes in gene expression accompanying altered auditory input have however, most often been investigated using total cochlear ablation. **Purpose:** We evaluated the effect of more realistic, limited lesions of the cochlear epithelium on the spontaneous firing rates of midbrain neurons and on the expression of genes related to synaptic function and neuronal excitability. **Methods:** Anaesthetized guinea pigs (n=5) received a small mechanical lesion in the left organ of Corti and were allowed to recover for 1 week. They were re-anaesthetized and recordings were made from single cells in the contralateral inferior colliculus before processing tissues for mRNA levels of 10 genes. Expression was compared with a group of unlesioned animals (n=5). **Results:** Spontaneous hyperactivity was consistently found in the auditory midbrain 1 week after cochlear lesions. Expression levels of TASK5 (potassium leakage channel) and genes related to GABAergic neurotransmission were reduced in both inferior colliculus and cochlear nucleus ($P<0.05$). Glycine receptor expression was depressed in cochlear nucleus only. Changes in expression in inferior colliculus were bilateral whereas those in cochlear nucleus were only found ipsilateral to the lesioned cochlea. **Conclusions:** altered input from a restricted region of the cochlea results in increased excitability in auditory midbrain. This is accompanied by reduced expression of genes related to inhibitory neurotransmission and stability of neuronal membrane potential. The distribution of ipsilateral and bilateral changes in cochlear nucleus and inferior colliculus respectively is consistent with the anatomical organization of the ascending pathway.

POS-WED-239

DETERMINING IMPORTANT MEDIATORS OF INFLAMMATION AND NEUROTOXICITY IN ALZHEIMER'S DISEASE USING HUMAN IN VITRO MODELS

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Purpose: Our previous studies using isolated cultures have shown that Alzheimer's disease (AD) associated Aβ oligomers are more potent stimulators of inflammation compared to their fibrillar counterparts. The present study examined the effect of oligomeric $A\beta_{42}$ on inflammation and neurotoxicity using a more complex, triple-culture model consisting of human astrocytes, neurons and microglia. **Methods:** Cultures were stimulated with recombinant 10μM oligomeric $A\beta_{42}$ for 16 and 48 hours (n=3/group). Conditioned media was assessed for the presence of 17 cytokines and chemokines using the Bio-Plex™ bead-based immunoassay and significant differences between groups were determined by multivariate tests using Wilks' Lambda. Neurotoxicity was assessed using a lactate dehydrogenase assay and total levels/phosphorylation of tau assessed by Western blotting with group differences determined by non-parametric Kruskal-Wallis and Mann Whitney U post hoc tests. **Results:** Treatment with oligomeric $A\beta_{42}$ caused minimal neuronal death in triple-cultures compared with neurons cultured in isolation ($p=0.035$). Neuronal survival was associated with sustained increases in IL-1β, IFN-γ, GM-CSF, IL-8 and MIP-1β secretion over time ($p<0.05$), with TNF-α, IL-2, IL-4 and IL-12 initially increasing at 16 hours ($p<0.05$), and decreasing at 48 hours ($p>0.05$). Neuronal survival was also associated with increases in total tau levels at 48 hours (220 ± 55%, $p=0.019$). **Conclusion:** These data demonstrate a neuroprotective role against oligomeric $A\beta_{42}$ toxicity for TNF-α, IL-2, IL-4 and IL-12 with associated increases in IL-1β, IFN-γ, GM-CSF, MIP-1β and IL-8 stimulating neuronal tau transcription. The changes observed in these triple-cultures have similarities with data obtained from patients with mild cognitive impairment suggesting that this model may represent early disease processes.

POS-THU-240

INVOLVEMENT OF THE KYNURENINE PATHWAY IN ALZHEIMER'S DISEASE

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The kynurenine pathway (KP) of tryptophan (TRP) metabolism is one of the major regulatory mechanisms of the immune response. We have shown that this pathway is switched on in the neuroinflammatory process of Alzheimer's disease (AD) and is likely to be involved in the progression of AD pathogenesis. We initially demonstrated *in vitro* that Aβ1-42 led to significant production of the excitatory neurotoxin quinolinic acid (QUIN) by human primary macrophages and microglia. We believe that QUIN is subsequently involved in several neurodegenerative processes. Using immunohistochemistry we then found that QUIN is over-produced and the regulatory enzyme of the pathway, indoleamine 2,3 dioxygenase (IDO) is over-expressed within AD brain. Both IDO and QUIN were detected in microglia, astrocytes and neurons within cortical sections of cortex. Microglial and astrocytic expression of IDO and QUIN was highest in the perimeter of senile plaques, which were also diffusely labelled. Within the neuronal cell body in AD tissue, QUIN was present in granular deposits and was also seen in uniform labelling of neurofibrillary tangles (NFT) and interestingly QUIN and hyperphosphorylated tau strongly co-localized. Finally, we found recently that *in vitro* biologically relevant concentrations of QUIN induce hyperphosphorylation of tau in cultured human neurons leading to NFT formation. We have accumulated evidence that QUIN plays an important role in the complex and multi-factorial cascade leading to neurodegeneration in AD. These results will open a new therapeutic door for AD patients, as KP inhibitors are already available.

POS-WED-241

GLYCOSAMINOGLYCANS INFLUENCE AGGREGATION OF AN AMYLOIDOGENIC TRANSTHYRETIN MUTANT (L55P TTR)

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Transthyretin (TTR) is a 55 kDa tetrameric protein containing 4 identical subunits. TTR is responsible for carrying the hormone thyroxine (T_4) and retinol binding protein. In familial amyloidotic polyneuropathy (FAP), mutations in TTR destabilise the native tetrameric state, and induce TTR to aggregate and form deposits in peripheral nerves. This accumulation of TTR ultimately leads to a peripheral neuropathy. Many FAP mutations in TTR have been described, with the most common being V30M. Previous studies have shown glycosaminoglycans (GAGs) closely associated with TTR deposits in post-mortem tissue of FAP patients. However, the role GAGs play in the pathogenesis of FAP is unclear. In this study, the effects of GAGs on aggregation of L55P mutant TTR, a highly amyloidogenic TTR variant, were assessed using dynamic light scattering. Our findings show that the rate of aggregation of L55P TTR was higher in the presence of heparin, chondroitin sulfate A and dermatan sulfate. Chondroitin sulfate C had a biphasic effect on L55P aggregation, as it stimulated aggregation at low concentrations and inhibited aggregation at higher concentrations. These findings support the hypothesis that GAGs may be involved in the aggregation and deposition of TTR in FAP, and may be useful in the development novel therapeutic compounds, which modify the aggregation and deposition of TTR. Furthermore, this study has implications in the study of other amyloidogenic proteins, including the β-amyloid protein of Alzheimer's disease.

POS-THU-242

MECHANISMS OF AMYLOID-INDUCED CALCIUM DYSREGULATION AND THE ROLE OF SYNAPTIC SCALING IN THE PROGRESSION OF ALZHEIMER'S DISEASE

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Although build-up of β-amyloid protein (Aβ) can lead to Alzheimer's disease (AD), the mechanism of Aβ neurotoxicity remains unclear. Calcium is an important mediator of synaptic plasticity and neuronal excitability, and it is likely that calcium dysregulation underpins the synaptic dysfunction that is observed in the hippocampus and cortex of AD brains. The mechanism that drives disease progression also remains unclear, but may be due to homeostatic synaptic scaling, which compensates for loss of synaptic activity by increasing the excitability of networked neurons. This increased excitability in healthy neurons would cause raised intracellular calcium, and thereby, increased susceptibility to amyloid toxicity. We used single-channel calcium imaging to examine the mechanism of calcium dysregulation in cortical neurons treated with amyloid proteins, with and without mediators of synaptic scaling, such as TNFα. We found that acute application of $A\beta_{42}$ induced a rapid increase in intracellular calcium, and that this response was reduced in magnitude as $A\beta_{42}$ was aged for 5h. After 24h, the calcium response to $A\beta_{42}$ was abolished. We found that as the aggregate size increased, the ability to induce a calcium response was reduced. In contrast, $A\beta_{40}$ induced a maximal response only after aging. Preliminary data suggests that TNFα moderates the effect of $A\beta_{42}$ on intracellular calcium in hippocampal neurons, decreasing the calcium response by 14% (n=4) after 15min TNFα, and by 33% (n=4) after 24h TNFα. Thus, our data support the hypothesis that the progression of AD is caused by amyloid-induced calcium dysregulation and the compensatory mechanisms of synaptic scaling.

POS-WED-243

BASAL FOREBRAIN CHOLINERGIC NEUROAL DENERVATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Degeneration of the cholinergic neurons of the basal forebrain, particularly of neurons that innervate the cortex and hippocampus, is a key feature of Alzheimer's disease. This disease feature is recapitulated in animal models of Alzheimer's disease, including in an acute model whereby amyloid beta (Aß) peptides are injected into the hippocampi of adult mice. We have shown that there is significant loss of acetyl choline transferase immunoreactivity in the basal forebrain 14 days following injection of 10nmol of human oligomeric Aß1-42 into the hippocampus, and that this degeneration is mediated by expression of the neural cell death receptor, p75 neurotrophin receptor (p75NTR, [1]). Although application of Aß1-42 to hippocampal slices has been shown to result in immediate changes to synaptic plasticity [2], we observed altered behaviour using a contextual fear conditioning paradigm in mice that had been injected with Aß1-42 14 days previously but not in those injected 5 days earlier (n=8). More surprisingly, Aß1-42-injected animals displayed increased freezing in the fear-conditioned context compared to control Aß1-16-injected mice, suggesting enhanced rather than impaired memory. Although Aß is best known for its neurotoxic properties, we have shown that oligomeric Aß1-42 can also stimulate adult neurogenesis and that cholinergic innervation to the hippocampus may suppress hippocampal neurogenesis. We are continuing to investigate the time course of cholinergic degeneration and whether an increase in hippocampal neurogenesis, though a combination of direct and indirect longer-term influences of Aß, can account for our behavioural results. [1] A Sotthibundhu et al., J Neurosci 2008 [2] DJ Selkoe, Behav Brain Res 2008.

POS-THU-244

TPD-43 STAINING IN ALZHEIMER DISEASE AND DEMENTIA WITH LEWY BODIES – EVIDENCE FOR DUAL PATHOLOGY?

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Purpose: The TAR DNA binding protein-43 (TDP-43) is a major component of the unbiquitinated inclusions in tau-negative frontotemporal lobar degeneration (FTLD). However, several authors have reported TDP-43 staining in Alzheimer disease (AD) and other neurodegenerative diseases. What is not known is whether this represents dual pathology in these patients or whether TDP-43 staining is not specific to FTLD. **Methods**: Hippocampus and frontal cortex sections from 81 patients with dementia onset >65 years and 24 age-matched controls were obtained from POWMRI-TRC. Sections were stained with antibodies to TDP-43 (1:500: ProteinTech), tau (AT8; 1:10,000; Pierce-Endogen) and alpha-synuclein (0.13mg/L; Zymed) and the number of neuronal cytoplasmic inclusions (NCIs), dystrophic neurites and neuronal intranuclear inclusions (NIIs) determined in the dentate gyrus, hippocampal CA1 sector, entorhinal and frontal cortices. **Results**: Fourteen of 43 AD (33%) and four of 21 Dementia with Lewy bodies (DLB) cases (19%) had coexisting TDP-43 pathology. In the majority of AD cases, TDP-43 staining was sparse and predominantly in the dentate gyrus. However, in three cases TDP-43 was prominent and coexisted with frontal atrophy and marked cortical neuron loss suggesting dual pathology. In one additional case TDP-43 staining of neurofibrillary tangles was apparent. In DLB two of the four TDP-43-positive cases had moderate numbers of NCIs and neurodegeneration suggesting dual pathology. AD cases with TDP-43 staining did not differ in age at death from cases without TDP-43 staining, however TDP-43-positive DLB cases were significantly older. Rare NIIs were found in three FTLD and one DLB case. **Conclusions**: While sparse TDP-43 staining is seen in one third of AD cases, dual pathology is apparent in a minority of both AD and DLB. These results have implications for the neuropathological classification of dementia cases and suggest that further elucidation of the thresholds of pathology is required for accurate diagnosis.

POS-WED-245

IMMUNE RESPONSES IN TAU-POSITIVE AND TAU-NEGATIVE FRONTOTEMPORAL LOBAR DEGENERATION

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Purpose: Microglial activation is a key feature of all Frontotemporal Lobar Degeneration (FTLD) subtypes, but the stimulus for this response is unknown. Complement proteins are capable of activating microglia, and have been previously identified in cases of Pick's Disease (PiD). The absence of Factor B in these cases suggests activation of the classical complement pathway, which is initiated by IgG or IgM. **Methods:** Sections of inferior temporal cortex from 32 cases of FTLD (including 13 PiD, 8 Corticobasal Degeneration (CBD), and 11 FTLD-U) and 6 age and gender matched control cases were obtained from the POWMRI-TRC. These were stained with anti-sera to HLA-DR (1:200, Dako), CD68 (1: 50, Dako), IgG (1:40, Serotec) and IgM (1:500, Serotec). The number of IgG and IgM positive neurons, and HLA-DR-positive and CD68-positive microglia were quantified. The neuronal density was also determined in each case. **Results:** The greatest number of IgG-positive neurons was evident in cases of FTLD-U, and a positive correlation between IgG-positive neurons and activated cortical microglia was observed ($p = 0.0058$) suggesting the microglial response is in part due to immunoglobulin binding. The number of IgG-positive neurons increased as the neuron density decreased ($p = 0.0078$) suggesting the immunoglobulin response is progressive over the course of the disease. **Conclusions:** An immune response may contribute to the neuroinflammation that is prominent in FTLD. There may be differences in the immune response between cases of tau-positive and tau-negative FTLD, which has implications for potential therapeutic strategies.

POS-THU-246

MALE HYPOGONADAL MICE HAVE ALTERED EXPRESSION OF ALZHEIMER'S DISEASE-RELATED PROTEINS

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Purpose: Evidence suggests that the depletion of sex hormones with age could be related to the onset or progression of Alzheimer's disease. It was the purpose of this study to examine the relationship between sex hormones and proteins linked to Alzheimer's disease (AD) using hypogonadal (hpg) mice that have no detectable levels of circulating sex hormones. **Methods:** Western blots and immunohistochemistry were used to analyze expression of proteins related to AD including amyloid precursor protein (APP), presenilin 1 (PS1), c-terminal fragment of APP (APP-CTF) and cholinergic acetyltransferase (ChAT) in wildtype/heterozyogous (n=8) and homozygous hpg mice (n=10) aged 12-18 months. **Results:** Depletion of sex hormones altered protein expression specifically in the male hippocampus with significantly lower APP ($p<0.01$), higher PS1 ($p<0.05$) and higher APP-CTF ($p<0.05$) in hpg hippocampus compared to age-matched wild-type hippocampus. There was no difference between hpg and wild-types in any other area of the brain or in the female hippocampus. Immunohistochemistry data revealed that these differences in hippocampal APP expression were also region specific in that male hpg mice had increased expression of APP in the dentate gyrus compared to wild-types, while there was no difference in APP expression in CA3. Male hpg mice also showed reduced ChAT immunoreactivity in the basal forebrain compared to wild-type mice, again with no difference evident in female brains. **Conclusion:** These results suggest that depletion of sex hormones may alter amyloidogenic processing of APP in the male hippocampus, similar to what is seen in AD. Hpg mice may therefore provide a useful alternate model in which to further examine the relationship between sex hormones and AD-related proteins.

POS-WED-247

MICROGLIAL-INDUCED NEUROTOXICITY AND ANTI-INFLAMMATORY NEUROPROTECTION

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Purpose: β-amyloid plaques, surrounded by activated microglial and astroglial cells, are found in the brains of Alzheimer's disease patients. Chronic over stimulation of these microglial cells in the brain is suggested to be one of the major causes of the slow, but constant loss of neurons associated with Alzheimer's disease. Microglial cells therefore might provide a target for the long term prevention of the disease. **Methods:** We developed a novel co-culture assay with GFP-expressing neurons, which allows to distinguish between microglial and neuronal viability. In this system, activation of microglia by IFNγ and LPS caused a dose- and time-dependent degeneration of neurons. This assay was then used to determine the neuroprotective properties of non-steroidal anti-inflammatory drugs, statins and complementary anti-oxidant medications (n=12). In a 96-well format, co-cultures were incubated for 12 hours with compounds, prior to 48 hours of activation with 1U/ml IFNγ and 1µg/ml. Relative neuronal cell numbers were then determined by measurement of GFP fluorescence with a Wallac Victor V fluorimeter. **Results:** Apigenin, Diosmetin, Coenzyme Q10, Indomethacin and inhibitors of Nitric Oxide Synthase increased neuron viability in a dose-dependent manner. The increase in neuronal viability caused by the drugs was correlated to their ability to decrease nitric oxide production. **Conclusion:** Natural anti-oxidant compounds including Coenzyme Q10, Apigenin and Diosmetin provide greater neuroprotection against microglial insults than several non-steroidal anti-inflammatory drugs and statins. Moreover, the neuroprotective potential of these compounds is linked to the inhibition of Nitric oxide production, suggesting that NOS inhibitors and anti-inflammatory antioxidants could be promising drugs for the treatment of diseases characterized by chronic inflammation including Alzheimer's disease.

POS-THU-248

LIPOIC ACID AS AN ANTI-INFLAMMATORY AND NEUROPROTECTIVE DRUG FOR ALZHEIMER'S DISEASE

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Purpose: The aim of this study was to investigate whether lipoic acid (LA) could down-regulate the expression of pro-inflammatory markers *in vitro* and *in vivo*, and whether it could slow down the progression of Alzheimer's disease (AD). **Methods:** The effects of LA on the expression of TNF and iNOS were measured in activated N11-microglia cells, neuroprotective properties of different LA enantiomers and dihydrolipoic acid (DHLA) against H_2O_2 insult were determined in Neuro2a cells. Furthermore, 600 mg rac-LA was given daily to patients with AD (n = 43; receiving a standard treatment with cholinesterase inhibitors) in an open-label study over an observation period of up to 48 months in a clinical study at the Henriettenhospital in Hannover, Germany. **Results:** In *in vitro* experiments we could show that LA significantly down-regulates the expression of redox-sensitive pro-inflammatory markers, including TNF and iNOS in N-11 microglia cells. Furthermore, LA reduced H_2O_2-induced cell death in Neuro2a cells significantly ($n=6$; $p < 0.05$), whereby both the R- and the S-isomer were equivalent. Patients with moderate dementia that received LA showed no significant improvement, while the disease progressed approximately at half the rate (change in ADAScog: 1.2 points/year, MMSE: - 0.6 points/year) in patients with mild dementia (ADAScog < 15) than in the control group treated only with cholinesterase inhibitors (n=12). **Conclusion:** These data suggest that LA reduces inflammation *in vitro* and *in vivo*, slows down the progression of early stage AD, and might become a promising new drug to treat early stages of AD.

POS-WED-249

THE INVOLVEMENT OF THE KYNURENINE PATHWAY AND INFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron degenerative disease. The kynurenine pathway (KP) catabolizes tryptophan (TRP) and generates neuroactive compounds, such as picolinic acid (PIC) and quinolinic acid (QUIN), is emerging as a possible pathogenic component of ALS. Indoleamine-2,3 dioxygenase (IDO) is the first enzyme in the KP. **OBJECTIVES**: This study aims to characterize the KP in ALS patients and the NSC-34 motor neuron cell line; and assess the effect of QUIN toxicity on NSC-34 cells. **METHODS**: Using GC/MS and HPLC, CSF and serum QUIN, PIC, TRP and kynurenine levels of ALS patients (n=150) and controls (n=20) were analyzed. Antibodies to HLA-DR, IDO and QUIN were used on paraffin embedded ALS human spinal cord and motor cortex sections. In NSC-34 cells, RT-PCR and immunocytochemistry were used to characterize KP enzymes and catabolites; LDH test assessed the effect of QUIN, with and without inhibitors. **RESULTS**: In ALS samples, significant increases in CSF and serum TRP ($P<0.0001$), KYN ($P<0.0001$) and QUIN ($P<0.05$), and decrease serum PIC ($P<0.05$) were observed. Significant numbers of activated microglia expressing HLA-DR ($P< 0.0001$) and increases in neuronal and microglial expression of IDO and QUIN were detected in ALS motor cortex and spinal cord. NSC-34 cells stained positive for KP enzymes and catabolites; RT-PCR showed the presence of KP enzymes; and LDH production displayed a dose-dependant increase with QUIN, partially inhibited by antagonists. **CONCLUSION**: Our results provide the first strong evidence *in vitro* and *ex vivo* for the involvement of the KP in ALS.

POS-THU-250

GAS6 DEFICIENCY INCREASES DAMAGE BUT PROMOTES RECOVERY IN RESPONSE TO CUPRIZONE-INDUCED DEMYELINATION

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Multiple sclerosis (MS) is the commonest cause of neurological disability that affects young, Caucasian adults. MS is thought to result from an inflammatory or immune attack against myelin, which is produced by specialised glial cells known as oligodendrocytes. Here we describe the role of the TAM family of protein tyrosine kinase receptors, and their common ligand, Gas6, during cuprizone-mediated demyelination in mice. We have previously shown that Gas6 deficiency increases oligodendrocyte loss and microglial activation after three weeks of cuprizone challenge[1]. We hypothesised that TAM receptor signalling would also influence the extent of recovery in mice after withdrawal of cuprizone. Three groups of 8 week old Gas6 knockout and wild-type mice (n=3/4 per group per genotype) were subjected to cuprizone challenge for 5 weeks. One group was analysed at this time-point to provide nadir levels of myelination and oligodendrocyte numbers. The remaining mice were analysed at either two weeks or four weeks post-cuprizone withdrawal. As we have previously shown, in Gas6 knockout mice subjected to cuprizone, demyelination was greater than in control mice, notably in the more rostral regions of the corpus callosum (myelination reduced by ~27%) as assessed by both luxol fast blue staining and ultrastructure. However, by two weeks post-cuprizone withdrawal there was no significant difference between genotypes ($p>0.05$), suggesting that remyelination occurs effectively in the absence of Gas6, despite the greater extent of the initial damage. We are currently assessing levels of oligodendrocyte progenitor recruitment, microglial activation, and completing a detailed ultrastructural analysis of myelin integrity in these mice. 1. Binder et al (2008). *J.Neurosci.* 8(20):5195-206.

POS-WED-251

DISABLED-2 (DAB2) IS IMPLICATED IN THE PATHOGENESIS OF EAE

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Purpose: We previously identified Dab2 as a novel gene that is up-regulated in response to the animal model of multiple sclerosis (MS) experimental autoimmune encephalomyelitis (EAE), relative to healthy controls. In this study we assessed the functional significance of the loss of Dab2 both in health and in EAE. **Results:** Assessment of mice in which Dab2 was conditionally deleted from the whole embryo, but not extra-embryonic tissue (Meox2Cre), revealed that mice heterozygous or homozygous for the Dab2 deletion were developmentally normal with no differences in CNS morphology or cell numbers relative to wild-type littermates (n=4 per genotype). Conditional deletion of the Dab2 gene resulted in reduced EAE disease severity in mice heterozygous for Dab2, statistically significant from day 16 post-induction onwards ($p <0.01$, n= 15 wild-type, and n=24 heterozygous mice). Preliminary data further showed that mice homozygous for the Dab2 deletion had delayed EAE disease onset. **Conclusions:** These results indicate that Dab2 is likely to have a role in the disease pathogenesis of EAE. Given that it is not expressed by lymphocytes, but at high levels in microglia, this suggests that it might be involved in modulating the innate disease response. We will explore this possibility utilizing cell culture and further in-vivo analyses.

POS-THU-252

THE MBP-DTR MOUSE: A MODEL OF INDUCIBLE OLIGODENDROCYTE APOPTOSIS IN THE ADULT BRAIN

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Purpose: Oligodendrocyte apoptosis is among the earliest neurohistopathological features of multiple sclerosis. To study the cellular neuropathological sequelae, a model of inducible oligodendrocyte apoptosis is required. Here we describe the generation of transgenic mice in which oligodendrocytes are rendered selectively sensitive to Diphtheria toxin (DT)-mediated apoptosis. **Methods:** A plasmid construct comprising the coding sequence for Diphtheria toxin receptor (DTR) under the regulatory control of the proximal promoter for murine myelin basic protein (MBP) was microinjected into fertilised C57BL/6 eggs. Resultant MBP-DTR transgenic lines were assessed for DTR expression and for oligodendrocyte integrity, myelination and inflammatory responses following DT administration. **Results:** Several MBP-DTR lines have been generated, with DTR expression restricted to mature oligodendrocytes. Expression levels vary between lines, ranging from 100% of oligodendrocytes to <1%; initial DT administration experiments have utilised a line in which 25% of oligodendrocytes express DTR. Administration of DT to MBP-DTR mice results in oligodendrocyte apoptosis, loss of DTR immunoreactivity, and oligodendrocyte loss after 5 days (n=1). Three weeks after DT administration, MBP-DTR mice exhibit clinical disability, profound CNS inflammation, and some demyelination (n=1). Prominent astrogliosis and microglial activation in grey matter suggest neuronal/axonal injury, this is currently under investigation. **Conclusions:** Administration of DT to transgenic animals expressing DTR on mature oligodendrocytes results in the selective ablation of these cells. Consequences of oligodendrocyte death include demyelination and widespread inflammation. Additional effects of oligodendrocyte death are under investigation. This model will be of significant value in understanding the molecular and cellular consequences of oligodendrocyte death, and for defining new strategies to enhance repair.

POS-WED-253

MODULATION OF BONE MORPHOGENIC SIGNALLING DURING DEMYELINATION

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Purpose: Bone morphogenic proteins (BMPs) inhibit oligodendrogenesis by decreasing neural precursor cell (NPC) proliferation and oligodendrocyte production. We have previously shown that BMP4 is upregulated in the subventricular zone (SVZ) during demyelination. Here, we examine effects of modulating BMP signalling on NPC gene expression and proliferation and lineage commitment during demyelination. **Methods:** Gene expression profiling was performed on primary cultures of adult SVZ NPCs following BMP4 or Noggin application using QPCR Signalling Arrays. For demyelination studies, BMP4, Noggin or vehicle were infused into the lateral ventricle by micro-osmotic pumps during cuprizone induced demyelination. Proliferation was identified by incorporating BrdU (1mg/mL) into drinking water. BrdU and lineage specific proteins were detected using immunohistochemistry. **Results:** Gene expression profiling revealed that neurogenic and oligodendroglial responsive genes were differentially regulated by BMP4 and Noggin. Immunohistochemistry showed increased BMP signalling in the SVZ and corpus callosum (CC) (n=4,4; p<0.01) during demyelination as observed by phosphorylated SMAD1/5/8 immunoreactivity. We found that p-SMAD 1/5/8 co-localised primarily with GFAP+ astrocytes in the SVZ, whereas, in the CC, p-SMAD 1/5/8 and Olig2 double positive cells were significantly increased (n=4,4; p<0.001). Intraventricular infusion of BMP4 increased p-SMAD 1/5/8 immunoreactivity while Noggin infusion decreased immunoreactivity in the SVZ and CC. **Conclusion:** These findings demonstrate that BMP signalling affects adult SVZ NPCs neurogenic and oligodendroglial gene expression, and is increased in the SVZ and CC during demyelination. Modulating the BMP pathway *in vivo* resulted in p-SMAD 1/5/8 being differentially regulated with BMP4 or Noggin infusion. We are currently examining changes in proliferation and lineage commitment associated with BMP modulation during demyelination to determine whether inhibition of BMP signalling during demyelination increases oligodendrocyte production.

POS-THU-254

THE COMPLEMENT FACTOR C5A CONTRIBUTES TO PATHOLOGY IN A RAT MODEL OF MOTOR NEURON DISEASE

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Purpose: Complement activation products are elevated in the cerebrospinal fluid and spinal cord of patients with motor neuron disease (MND). Recent studies have also shown the classical complement system factor C1q is upregulated in mouse SOD1 models of MND. This study examined the involvement of the potent complement inflammatory factor C5a in a rat model of MND. **Methods:** Lumbar spinal cords of transgenic $SOD1^{G93A}$ rats were examined for the degree complement activation (C3b deposition) and expression of the receptors for C5a; C5a receptor (C5aR) and C5a-like receptor 2 (C5L2). $SOD1^{G93A}$ rats were also treated with the selective C5aR antagonist, PMX205 (1mg/kg/day) from an early age (P28). **Results:** C5aRs were strongly expressed on motoneurons from wild-type rats (n=4). At end stage disease, $SOD1^{G93A}$ rats displayed marked deposition of C3/C3b, and a significant upregulation of C5aR specifically on proliferating astrocytes (n=4). The expression of C5L2, the alternative receptor for C5a, was highest on motoneurons early in the disease process (P90, n=5). $SOD1^{G93A}$ rats treated with PMX205 displayed a significant extension of survival time (127 ± 4 days vs 144 ± 8 days; n=8-12) and a reduction in end-stage motor scores, as compared to vehicle-treated rats. PMX205-treated animals also displayed reduced levels of astroglial proliferation in the lumbar spinal cord (n=5). **Conclusions:** This study demonstrates an involvement of C5a in an MND rat model, and suggests that inhibitors of complement activation could be beneficial in the treatment of this neurodegenerative disease.

POS-WED-255

THROMBIN ACTIVATION OF ASTROCYTES INDUCES MORPHOLOGICAL CHANGES AND CELL INJURY IN MIXED RAT HIPPOCAMPAL CULTURES

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Purpose: Thrombin, a blood protease well known for its role in coagulation and platelet activation, is also reported to influence cells in the CNS. It is implicated in neurodegenerative processes *in vivo* and can induce a variety of neuronal and non-neuronal responses *in vitro*. We investigated the mechanisms by which thrombin affects rat hippocampal cultures. **Methods:** Rat-pup primary hippocampal cultures, comprising of ~40% neurons and ~60% astrocytes, were exposed to thrombin (10 U/ml) for 48-72hr or to the PAR-1 agonist, TRAP (SFLLRN; 100μM). Cultures were imaged by phase-contrast microscopy, immunostained for neurons and astrocytes and tested for viability. Changes in intracellular calcium flux ($\Delta[Ca^{+2}]_i$) were also recorded during thrombin and NMDA perfusion. **Results:** Thrombin treatment resulted in the formation of cell-free areas (termed "craters") in the culture monolayer, which was blocked by the thrombin inhibitor, Hirudin (10 ATU/ml). Immunocytochemistical analysis further revealed a dramatic rearrangement of the astrocytic layer, with neurons, but not astrocytes, being "trapped" within craters after thrombin stimulation. Treatment of cells with TRAP also led to crater formation indicating that the effect of thrombin was via PAR-1 signaling. Thrombin-induced crater formation coincided with a reduction in cell viability at 48hr (P<0.05). The sensitivity of cultures to thrombin was maintained following prior NMDA-induced neuronal injury, suggesting that non-neuronal cells are the major target for thrombin. Consistent with this, thrombin elicited a sharp rise in $\Delta[Ca^{+2}]_i$ in non-NMDA responsive cells, but not in NMDA-responsive neurons. **Conclusion:** In mixed hippocampal cultures, thrombin activation of PAR-1 on non-neuronal cells, primarily astrocytes, triggers a cascade of morphological changes which leads to neuronal loss. Hence this study identifies astrocytes as key mediators of harmful thrombin signals in the hippocampus.

POS-THU-256

INCREASED ENDOGENOUS TISSUE TYPE-PLASMINOGEN ACTIVATOR (T-PA) CORRELATES WITH INCREASED MATRIX METALLOPROTEINASE-9 ACTIVATION FOLLOWING TRAUMATIC BRAIN INJURY

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Purpose: To investigate the role of tissue-type plasminogen activator (t-PA) in secondary damage following traumatic brain injury (TBI). t-PA is a serine protease that plays an important role in fibrinolysis, via its conversion of plasminogen to plasmin. Under neuropathological conditions such as stroke and TBI, t-PA is known to exacerbate blood-brain barrier (BBB) breakdown, primarily via its induction of matrix metalloproteinase-9 (MMP-9) expression. **Methods:** We used the controlled cortical impact (CCI) model to induce TBI in wildtype, t-PA -/- and t-PA transgenic mice. We analysed the damage to the BBB at 1.5 h, 3 h and 24 h post-trauma in these mice (n=6 each) compared to sham animals of each genotype, to determine whether increased endogenous t-PA in the transgenic mice would make them more susceptible to BBB breakdown. We used gelatin zymography, western blotting and immunohistochemistry techniques to characterise MMP-9 activity, and reverse transcriptase PCR (RT-PCR) to characterise changes in MMP-9 expression levels in brain tissue harvested from these mice. **Results:** We detected increased MMP-9 activity as early as 3 h post-trauma in the t-PA transgenic mice and reduced MMP-9 activity in the t-PA -/- mice, when compared to litter-matched wildtype controls. These changes were not subsequent to an increase in MMP-9 gene expression at this time-point; in contrast, there were significant differences in the expression of the tissue inhibitor of metalloproteinase-1 or TIMP-1, an endogenous MMP-9 inhibitor. **Conclusions:** To our knowledge, this is the first description of augmented MMP-9 activity dependent on endogenous t-PA levels under neuropathological conditions *in vivo*. The mechanism by which t-PA modulates MMP-9/TIMP-1 activity and ensuing BBB breakdown is under investigation.

POS-WED-257

CHARACTERISING ENDOGENOUS NEUROGENESIS FOLLOWING EXPERIMENTAL FOCAL TRAUMATIC BRAIN INJURY (TBI)

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Purpose: Neurogenesis occurs in response to various types of brain injury. However, the induction of specific stages of neurogenesis has not been examined in experimental TBI models. This study aimed to characterise the neurogenic response in a closed head injury (CHI) model of focal TBI. **Methods:** Adult C57BL/6 mice were subjected to CHI or sham-operation. BrdU (200mg/kg i.p.) was administered twice-daily for 4d beginning 1d post-injury, to label proliferating cells. Brains were collected 1,2,4&8w post-trauma (n=4-5). BrdU-labelled cells were quantified in the dentate gyrus (DG) and the subventricular zone (SVZ), to assess cell *proliferation* and *survival*. Neuronal *differentiation* was assessed following BrdU co-labelling with DCX (differentiated/migrating neurons). Neuronal and glial *maturation/survival* was quantified as the %BrdU-labelled cells co-labelled with NeuN or GFAP. **Results:** CHI increased the number of new cells at all time-points up to 3-fold in the DG ($P<0.001$), and up to 2-fold in the SVZ ($P<0.05$). A reduction in new cells occurred at 4w post-CHI in the DG ($P<0.01$), and at 2w in CHI and controls in the SVZ ($P<0.05$). Abundant neuronal differentiation was evident at 1w in the DG and SVZ, with migrating cells observed in the corpus callosum and pericontusional-cortex. At 4,8w post-injury, the majority of new cells in the DG expressed GFAP (~60%), while less expressed NeuN (~10%); however, the number of new neurons was not changed following CHI ($P>0.05$). In the pericontusional cortex, ~60% of BrdU-labelled cells were astrocytes, and very few new neurons were detected. **Conclusion:** Focal TBI induces proliferation in the DG and SVZ, however, this does not result in enhanced neuronal production. Future studies will investigate stimulating neurogenesis with specific factors to enhance neuronal differentiation and survival, potentially aiding recovery following TBI.

POS-THU-258

POST-TRAUMATIC HYPOXIA EXACERBATES BRAIN DAMAGE IN AN ANIMAL MODEL OF DIFFUSE AXONAL INJURY

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Purpose: Secondary systemic insults, such as hypoxia, have demonstrated to increase neurological deficit in severe TBI patients. In this study, we characterized the axonal damage, astrocytic and microglial activation, and cell death in rats subjected to traumatic axonal injury (TAI) with/without additional systemic hypoxia. **Methods:** TAI was induced by dropping a 450g weight from 2m onto a metal disc affixed to the skull. Rats were subsequently ventilated with either 12% O_2 (TAI+hypoxia) or 22% O_2 (TAI+normoxia) for 30min. Uninjured sham controls underwent surgery and normoxic ventilation. Rats were euthanised at 1d or 7d post-DAI (n=4/group/timepoint), and brains were perfusion fixed and wax embedded. Immunohistochemistry on 10µm sections was performed to determine axonal damage (APP, neurofilament), astrocytosis (GFAP), macrophage infiltration/microglial activation (CD68) and cell death (TUNEL) by peroxidase technique. **Results:** APP and neurofilament staining showed the swelling axons and retraction bulbs in the corpus callosum, the granular layer of the cortex, and the pyramidal tracts of the brainstem at 1d and 7d in the TAI+hypoxia group. In comparison, the TAI+normoxia group showed only the retraction bulbs at 1d and 7d but no axonal swelling. Infiltrated macrophages and activated microglia were localised to the corpus callosum (TAI+hypoxia: 3.685±0.345%; TAI+normoxia: 2.920±0.312%; sham: 1.13±0.18%) and the optic tract (TAI+hypoxia: 4.06±0.08%; TAI+normoxia: 2.45±0.11%; sham: 0.06±0.01%) at 7d. Astrocytes activation was detected throughout the brain parenchyma at 1d and 7d in TAI animals. Cell death was not abundant throughout the brain, however, TUNEL-positive cells were increased in the corpus callosum of TAI brains at 1d and 7d (TAI+hypoxia: 18.10±1.86 cells, 16.13±2.02; TAI+normoxia: 19.25±4.99, 13.17±3.42; respectively; sham: 7.00±1.04). **Conclusion:** This study demonstrates that systemic hypoxia following trauma increases axonal damage and glial activation, but did not increase cell death when compared to trauma only.

POS-WED-259

FAS MEDIATES TISSUE DAMAGE AND NEUROLOGICAL DEFICIT FOLLOWING FOCAL TRAUMATIC BRAIN INJURY

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BACKGROUND: Neuronal apoptosis contributes to ongoing tissue damage following traumatic brain injury (TBI). This study aimed to determine whether partial inhibition of the extrinsic pathway of apoptosis via genetic mutation of the Fas receptor would decrease lesion volume, cell death, and improve neurological outcome in a mouse model of focal TBI. **METHODS:** Mice expressing non-functional Fas receptor (*lpr*) were killed at 1, 4 and 7d post-TBI and compared to wild type controls (WT). Lesion volume was determined on H&E-stained cryosections while apoptotic cells were counted throughout the cortical contusion following TUNEL and caspase-3 immunohistochemistry. Neurological outcome was assessed at 1h and then every 24h post-TBI. **RESULTS:** Lesion volume and number of TUNEL+ cells were significantly reduced in *lpr* mice as compared to WT mice ($p=0.017$ and $p=0.011$, respectively, n=5/group). Numbers of TUNEL+ cells correlated with caspase-3+ cells at 24h (Spearman's rho=0.410, $p=0.005$), suggesting that TUNEL+ cells are apoptotic. Up to 50% of these apoptotic cells co-localised with NeuN, a marker for mature neurons, while 5-10% of the remaining apoptotic cells co-localised with GFAP (astrocytes) or F4/80 (microglia/macrophages). Double-labelling was also performed with a marker for oligodendrocytes, however very few of these cells were observed to co-localise with TUNEL staining. *Lpr* mice showed improved neurological outcome from 2d to 7d post-TBI ($p<0.05$, n=10/group). **CONCLUSION:** Fas mutant mice show improved neurological outcome following TBI, possibly due to reduced lesion volume and neuronal cell apoptosis, suggesting that therapeutic inhibition of Fas receptor may be the optimal neuroprotective strategy following TBI.

POS-THU-260

DEFICIENCY OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1/CCL2) IMPROVES LONG-TERM OUTCOME IN MICE FOLLOWING TRAUMATIC BRAIN INJURY (TBI)

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PURPOSE: The chemokine MCP-1/CCL2 functions by recruiting macrophages into inflamed tissues, and is implicated in several pathological conditions, including the secondary tissue damage seen as a result of TBI. This study aims to elucidate MCP-1's role following TBI by examining several parameters in MCP-1 knockout (KO) mice subjected to a focal closed head injury (CHI) model. **METHODS AND RESULTS:** Surprisingly, no differences in macrophage numbers (determined by F4/80 immunohistochemistry on wax-embedded sections) were found in the lesion site of MCP-1 KO mice compared to wildtype (C57Bl/6) controls at 4 or 7d post-CHI. However by 14 and 28d, there was a significant reduction in the spread of macrophages in KO brains (n=6-7, ~50%, $p<0.05$). This corresponded with a reduction in total lesion volume at 14 and 28d post-CHI in MCP-1 KO mice (n=6-7, $p<0.05$), as determined by H&E staining and NeuN immunohistochemistry to detect neuronal loss. Furthermore, neurological assessment (by a Neurological Severity Score and ledged beam test performance) revealed that MCP-1 KO mice had significantly improved recovery compared to wildtype mice from 10d until at least 28d post-CHI (n=8-9). By 28d the cortical lesion core in all mice appeared devoid of NeuN+ neurons and surrounded by GFAP+ reactive astrocytes. In addition, F4/80+ microglia were detectable in the ipsilateral thalamus. **CONCLUSIONS:** Further characterisation of this damage endeavours to better comprehend the mechanisms behind reduced tissue damage and subsequent improved neurological outcome observed in these MCP-1 KO mice. Thus far, this study suggests that MCP-1 may be detrimental to long-term recovery and contribute to exacerbation of secondary damage following TBI.

POS-WED-261

ULTRASTRUCTURAL CHANGES IN CHOROIDAL EPITHELIAL CELLS IN TRAUMATIC BRAIN INJURY

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Purpose: Elevation of intracranial pressure (ICP) is a major complication of traumatic brain injury (TBI), and cerebrospinal fluid (CSF) volume is a key factor in ICP regulation. Choroidal epithelial cells (CEC) form the blood-CSF barrier and are the main producers of the CSF. In the current study, the ultrastructure of the CEC was studied from 5 h to 28 d after TBI in the rat. **Methods:** This project was approved by the Animal Ethics Committee of the University of Adelaide. Male Sprague-Dawley rats (average weight 410g) were subjected to severe TBI (n=18) using the impact-acceleration model, and the ultrastructure of the CEC was studied using transmission (TEM) and scanning (SEM) electron microscopy. Three naive rats were used as controls. **Results:** Radical ultrastructural changes were seen by TEM in CEC in all injured animals. At 5 h post-injury cell swelling and incipient cytoplasmic vacuoles were seen. At 24h most severe changes were noted with extensive widening of intercellular clefts. At 7d and 14d post-injury, increased cytoplasmic electron density was evident. At 21d, most microvilli had bulbous ends, and at 28d cytoplasmic vacuoles were still present with widened intercellular clefts. SEM showed a continuum of changes in all injured animals and most conspicuous was the heterogeneity of surface features, with most cells showing bulbous and cup-shaped microvilli, burr-like processes and pits. Epiplexus cells were hypertrophic and more numerous. **Conclusion:** At 4 weeks after trauma, choroidal epithelial cells continued to show morphological alterations suggesting that brain homeostasis was still compromised.

POS-THU-262

EFFECTS OF HYPOXIA ON ICP AND BRAIN OXYGENATION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

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Purpose: Traumatic brain injury (TBI) causes a significant number of deaths, with survival often associated with permanent mental and physical disability. Poor outcome following TBI is coupled with secondary cerebral oedema and concomitant increases in intracranial pressure (ICP) for which there is currently no effective pharmacological treatment. The development of novel treatments would optimally involve the use of animal models which replicate the physiological response to TBI observed in humans. Whereas rodents have been the model of choice, and oedema formation has been demonstrated in rat models of TBI, no prior study has characterized the effects of TBI on ICP and brain tissue oxygenation ($P_{bt}O_2$). **Methods:** TBI was induced using the impact/acceleration model (n=13). ICP (Codman ICP Express) and $P_{bt}O_2$ (LICOX microprobe) were monitored for 4 hours. To simulate the effects of post-traumatic apnoea, a subgroup (n=6) of ventilated animals were subjected to 15 minutes of hypoxia (15% O_2, 85% N_2) immediately following injury, and subsequently returned to normoxic conditions (30% O_2, 70% N_2). **Results:** TBI alone did not result in an elevation of ICP, however $P_{bt}O_2$ was modestly reduced in these animals. The latter may have been related to the fall in arterial blood pressure. In contrast, TBI coupled with a hypoxic episode resulted in significant elevation of ICP and marked depression of $P_{bt}O_2$. **Conclusion:** Rats are a poor model for the characterization of ICP and $P_{bt}O_2$ in TBI unless injury is followed by a period of hypoxia. Novel therapies for the treatment of secondary cerebral oedema and elevated ICP should be explored in other species.

POS-WED-263

THE EFFECT OF A BETA-LACTAM ANTIBIOTIC CEFTRIAXONE ON GLUTAMATE TRANSPORTERS IN CA1 HIPPOCAMPAL REGION: IMPLICATIONS FOR NEUROPROTECTION

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Astrocytic glutamate transporters are considered an important target for neuroprotective therapies as the function of these transporters is abnormal in stroke and other neurological disorders associated with excitotoxicity. Recently, Rothstein et al. (2005) reported that beta-lactam antibiotics (including ceftriaxone, which easily crosses the blood–brain barrier) increase glutamate transporter 1 (GLT-1) expression and reduce cell death resulting from oxygen-glucose deprivation (OGD) in dissociated embryonic cortical cultures. To determine whether a similar neuroprotective mechanism operates in mature neurons, which show a different pattern of response to ischemia than primary cultures, we exposed acute hippocampal slices obtained from rats treated with ceftriaxone for 5 days (200 mg/kg; i.p.) to glutamate or OGD. Whole-cell patch clamp recording of glutamate-induced N-methyl-d-aspartate (NMDA) currents from CA1 pyramidal neurons showed a larger potentiation of these currents after application of 15 μM dl-threo-β-benzyloxyaspartic acid (TBOA; a potet blocker of glutamate transporters) in ceftriaxone-injected animals than in controls, indicating increased glutamate transporter activity. Western blot analysis did not reveal GLT-1 upregulation in the hippocampus. Delay to OGD-induced 'hypoxic spreading depression' recorded in slices obtained from ceftriaxone-treated rats was longer (6.3±0.2 vs. 5.2±0.2 min; P<0.001) than in controls, demonstrating a neuroprotective action of the antibiotic. Thus we confirm the neuroprotective effect of ceftriaxone on OGD-induced injury in acute hippocampal slices, but suggest that this action is related to modulation of transporter activity rather than to the level of GLT-1 expression. In separate experiments, ceftriaxone pre-treatment resulted in no neuroprotection against OGD or glutamate in hippocampal organotypic slice cultures. Our results indicate that the protective effects of β-lactam antibiotics are highly dependent on the experimental model.

POS-THU-264

DIFFERENTIAL EXPRESSION OF TRPV4 AND TRPM2 CHANNELS IN THE HIPPOCAMPUS: SIGNIFICANCE FOR ISCHEMIA-INDUCED CELL DAMAGE

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Transient receptor potential (TRP) channels are expressed in the CNS but their exact expression pattern and function are not fully understood. Considerable evidence suggests that TRPV4 and TRPM2 channels are involved in cell damage associated with various brain pathologies. TRPV4 are sensitive to low pH and cell swelling and are thought to be involved in brain ischemia. TRPM2 are activated by reactive oxygen species and therefore are likely to play a role in oxidative stress-induced cell death. We performed RT-PCR and Western blot analysis in acutely dissected rat hippocampi and organotypic hippocampal cultures. Expression of TRPV4 and TRPM2 was observed at mRNA and protein levels in both preparations (n=3). Immunocytochemistry demonstrated TRPV4 expression mainly outside the CA1/CA3 pyramidal and dentate granule cell layers. Double labeling for TRPV4 and GFAP revealed that TRPV4 immunoreactive cells were astrocytes. TRPM2-immunoreactive cells were found in the CA1/CA3 pyramidal and dentate granule cells. The immunostaining was colocalized with MAP2, demonstrating neuronal expression. Organotypic cultures (n=15) were subject to a short period of oxygen-glucose deprivation (OGD) and cell damage assessed 24 hrs later using the uptake of propidium iodide. Cell damage was mainly observed in the CA1 region which is TRPM2- immunoreactive, but not of TRPV4-expressing astrocytes. Endogenous increase of H2O2 production in slice cultures (n=29) was induced by inhibitor of glutathione oxidase (buthionine sulfoximine). This evoked damage of TRPV4-expressing astrocytes, while CA1/CA3 hippocampal neurons were less vulnerable. Thus TRPV4 and TRPM2 are differentially expressed in astrocytes and pyramidal neurons, suggesting distinct pathophysiological roles. Our data suggest that TRPV4-expressing astrocytes are more vulnerable to endogenous H2O2-induced oxidative stress than neurons, and that their oxidative damage is TRPM2-independent.

POS-WED-265

NADPH OXIDASE, ANGIOGENESIS AND FUNCTIONAL RECOVERY FOLLOWING ENDOTHELIN-1 INDUCED STROKE IN CONSCIOUS RATS

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NADPH oxidase-derived reactive oxygen species contribute to the progression of brain injury following stroke. Despite this, reactive oxygen species may also be crucial for optimal recovery through regulation of angiogenesis. **Purpose:** To identify in rat brain angiogenic factors, NADPH oxidase subunits, and blood vessel growth in the weeks following ischaemic stroke and reperfusion. **Methods:** The middle cerebral artery was constricted by endothelin-1 (ET-1) in conscious rats (n=24). Neurological and histological outcome was assessed by neurological deficit score and MCID image analysis. mRNA expression for Nox2, Nox4, and VEGF was assessed by qRT-PCR, 6h, 7, 14 and 28 days post-ET-1. Blood vessels were detected in situ using Von Willebrand Factor and point counted using Metamorph imaging software. **Results:** Significant deficits were detected between 1 and 7 days after ET-1 stroke but not after this time (P<0.01). Blood vessel numbers were decreased within the cortical infarct core 6 hours after stroke (29.7±10.3% P<0.05). By 28 days blood vessel numbers were markedly increased in the cortical infarct core (36.6±5.6%) and the cortical border zone (54.9±7.2%) in comparison to contralateral mirror regions (P<0.05). Angiogenic factor VEGF mRNA expression was increased in the ipsilateral cortex between 7 and 14 days post-stroke (~ 2 fold P<0.05) which later returned to normal levels by 28 days. Nox2 mRNA expression in the ipsilateral cortex was markedly increased up to 7 days post-stroke (up to 40 fold P<0.01) but returned to contralateral levels by 14 days. Nox4 mRNA was significantly increased at 14 days. **Conclusion:** Angiogenesis could be a target for promoting functional recovery post-stroke and this may involve Nox2 and Nox4 NAPDH oxidase.

POS-THU-266

INCREASED CORTICAL INFARCT AFTER TRANSIENT ISCHAEMIC STROKE IN MICE LACKING THE IP RECEPTOR FOR PROSTACYCLIN

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Purpose: Prostacyclin is a vasodilator, platelet anti-aggregatory and cytoprotective prostanoid generated mainly via the cyclooxygenase-2 (COX-2) pathway. Both enzyme and metabolites in the brain are upregulated following stroke. While inhibition of COX-2 reduces brain damage following ischaemic stroke, prostacyclin treatment also has documented protective actions. We have investigated whether endogenous prostacyclin plays a role in the brain damage following transient ischaemic stroke. **Methods:** Prostacyclin receptor-deficient mice (IP-/-; n = 7) on an apolipoprotein E-deficient background were compared to control littermates with functional IP receptor (IP+/+; n = 5) after occlusion of the middle cerebral artery (2 h) by an intraluminal filament. Following stroke superoxide generation was examined using dihydroethidium (DHE) fluorescence. **Results:** Following 24 h reperfusion, infarct volume was increased in the cerebral cortex of IP-/- mice (44.6 ± 9.6 mm^3) compared with IP+/+ littermates (10.0 ± 4.4 mm^3; $P \leq 0.01$), but not in the striatum. There was no difference in cerebral blood flow changes between groups as measured by laser Doppler flowmetry. Brain oedema tended to be increased in IP-/- mice but this was not significant. Compared to the appropriate contralateral control, brain sections from IP+/+ mice exhibited a decrease in core infarct DHE signal in the cortex (90 ± 5 %), whereas DHE fluorescence in the infarct of IP-/- mice was increased (122 ± 3 %). An increase in DHE fluorescence was detected in the ischaemic penumbra of both genotypes. **Conclusion:** The increased infarct size and oxidative stress in IP-/- mice indicates that endogenous prostacyclin signaling exerts a protective effect on cortical neuronal survival after transient stroke, but the mechanisms remain to be clarified.

POS-WED-267

MRI EVALUATION OF HYPOXIC-ISCHEMIC BRAIN INJURY IN THE NEONATAL PIGLET

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Purpose: Hypoxia ischemia (HI) remains a major cause of encephalopathy and neurodevelopmental disability in human neonates. Disruption to oxygen and glucose supply to the developing brain results in energy failure and elicits a cascade of biochemical events including excitotoxicity, inflammation and loss of ion homeostasis that culminates in neuronal dysfunction (seizures), cell injury and death. We aimed to investigate the value of early MRI evaluation of HI brain injury. **Methods:** Hypoxia (4% O_2) was initiated in term piglets (n=27) for 30 min. EEG was monitored to attain low amplitude EEG (<5 μV, LaEEG) and hypotension induced for the final 10 min. Daily EEG amplitude was recorded to determine seizure activity. Brain injury was assessed at 24 and 72 h using MRI and MRS. Animals were then euthanased and neuropathological injury analysed using histology. **Results:** Seizures were recorded in 78% of piglets. MRI and histological injury was greatest in HI animals with seizure activity ($p < 0.05$). There were significant differences in a number of MRS metabolite ratios at 24 ($p < 0.01$) and 72 h ($p < 0.05$) between HI animals with and without seizures and controls. There was good correlation of MR injury at both 24 and 72 h with histology ($p < 0.05$). **Conclusion:** Early monitoring of HI injury progression may aid not only prognosis of infants at risk of later neurodevelopmental disability but also in evaluation of treatment interventions for seizures.

POS-THU-268

A COMPARISON OF VISUAL IDENTIFICATION OF SEIZURES AND EEG DETECTION METHODS IN THE HUMAN NEONATE

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Purpose: Hypoxic brain injury due to perinatal asphyxia is a leading cause of morbidity and mortality in term neonates, although intrapartum detection of hypoxic insults is difficult. Treatment strategies are largely dependent upon early identification of high-risk infants. One of the major symptoms of underlying hypoxic brain injury is the presence of seizures; however neonatal seizures may present subclinically. We aimed to assess the incidence of seizures in hypoxic term neonates and report the level of agreement between seizure detection on 2-channel amplitude-integrated EEG (aEEG), standard multi-channel EEG and visually identified clinical seizures. **Methods:** 40 term neonates diagnosed with perinatal asphyxia were monitored continuously for 24-48 h using aEEG. Standard multi-channel EEG was performed between day 1 and 5 of life (1 h). Infants were assigned into good or poor outcome groups. **Results:** 38 enrolled neonates had follow-up information collected. There was no significant difference in peripartum parameters (cord pH, Apgar score, birth weight) between poor outcome and good outcome groups. 28 neonates had seizure detected on aEEG and 23 were treated with anticonvulsant. Only 7 neonates displayed seizure on multi-channel EEG. 31 neonates had documented clinical seizures, of which 29 received anticonvulsant treatment. 6/31 neonates were treated for clinical seizures with no evidence of aEEG/EEG seizure. **Conclusion:** Long-term monitoring is necessary to identify all seizure activity. While episodic multi-channel EEG is still considered the gold standard for seizure detection, continuous 2-channel aEEG is a reliable tool for detecting neonatal seizures.

POS-WED-269

EXOGENOUS ADDITION OF IN-HOUSE PURIFIED CYLOPHILIN A PROTEIN IS NEUROPROTECTIVE IN IN-VITRO NEURONAL CULTURE MODELS OF STROKE

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Cerebral ischaemia, a leading cause of death and disability, occurs when the blood supply to the whole or part of the brain is disrupted. The ensuing oxygen and nutrient deprivation causes neuronal loss, the extent of which correlates with patient injury and prognosis. Therapies to prevent this neuronal cell death are urgently needed. **Purpose:** We are evaluating the efficacy of several potentially neuroprotective proteins in rat cortical neuronal culture models of ischaemic (oxygen glucose deprivation; OGD), excitotoxic (glutamate exposure) and oxidative (cumene exposure) injury. One such protein, cyclophilin A (CyPA), which we previously reported is up-regulated by erythropoietin (EPO) and neuroprotective when over-expressed in neurons, was selected as a target for further drug development. This study aimed to establish if CyPA could protect neuronal cultures following addition of the purified protein to neuronal cultures. **Methods:** Recombinant CyPA protein was produced in E. coli (KRX strain) using the pET28a vector system, purified by His tag capture and assayed for enzyme activity and endotoxin. Cortical neuronal cultures (DIV 12) were exposed to OGD, glutamate or cumene. Recombinant CyPA protein was added to neuronal cultures either pre-, during- and/or post insult and neuronal viability was determined 24 hours post injury using the MTS assay. **Results:** We recovered soluble enzymatically active CyPA protein (>15mg/100ml). Exogenous addition of CyPA protein significantly increased the viability of neuronal cultures following OGD (n=1), glutamate (n=3) and cumene (n=3) exposure. **Conclusion:** Neuroprotection induced via the addition of exogenous CyPA protein is a novel and potentially viable treatment for cerebral ischaemia.

POS-THU-270

POST-ISCHAEMIC NEUROPROTECTION WITH MILD HYPOTHERMIA (35°C) AND MAGNESIUM FOLLOWING GLOBAL AND FOCAL CEREBRAL ISCHAEMIA IN RATS

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Purpose: Neurological deficits resulting from cerebral ischaemia have a profound impact on survivors of ischaemic stroke and cardiac arrest, yet there are few acute therapies available to reduce ischaemic brain damage. Many treatments have shown promise in animal models of cerebral ischaemia, though translating experimental successes to the clinic has been problematic. Improvements in preclinical studies, particularly in demonstrating successful intervention at clinically relevant delays to treatment, have been made to increase the opportunity for clinical trials to show a positive outcome. To this end, we have been assessing the neuroprotective efficacy of combined magnesium and mild hypothermia treatment when commenced after cerebral ischaemia in rats. **Methods:** Rats subjected to global (2 vessel occlusion + hypotension; 8 minutes) or focal (intraluminal thread, permanent middle cerebral artery occlusion) cerebral ischaemia were treated with 24 hours of mild hypothermia and/or a 24-48 hour infusion of magnesium sulphate. **Results:** Combination treatment, when commenced 2 hours after global ischaemia, increased hippocampal CA1 neuronal survival from 6% (saline control) to 76%, while mild hypothermia alone increased neuronal survival to 40%. After permanent focal ischaemia, the combination treatment decreased infarct volumes by 54% when started 2 hours after onset of ischaemia, whereas each treatment individually showed no effect. Treatment at 4 hours reduced infarct volumes by 39%, while by 6 hours the treatment benefit was lost. **Conclusion:** Combined treatment with magnesium and mild hypothermia is effective at reducing brain damage following cerebral ischaemia, even when treatment is delayed.

POS-WED-271

INFARCTION OCCURS MORE RAPIDLY IN HYPERTENSIVE RATS

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Purpose: We sought to determine the role of calcium-calmodulin-dependant protein kinase II (CaMKII) phosphorylation in cell survival or death after focal ischaemia using spontaneously hypertensive rats (SHR). Histological assessment of the brain 3 days after short occlusion periods unexpectedly found large infarcts. Therefore we undertook a study to determine the volume of infarction after varying durations of vessel occlusion in this rat strain in order to determine the optimum occlusion time to produce limited striatal injury without cortical infarction. **Method:** A well-established middle cerebral artery (MCA) thread-occlusion model of stroke was used in SHR, with occlusion times of 10, 15 or 20 minutes (n=5 each). Histological assessment was performed after 3 days survival. Infarct volumes were calculated, and comparisons were made to previous studies from our group and to published data. Where necessary, correction was made for volume loss due to fixation, by scaling to the volumes of the non-ischaemic cerebral hemispheres. **Results:** All experimental animals had measurable striatal infarct volumes following a 10min occlusion. After 20min MCAO there was substantial striatal and some cortical infarct in all animals. In contrast, published data from Wistar and our results in Sprague-Dawley rats do not show consistent infarction in these non-hypertensive strains with occlusion times of less than 25-45min. **Conclusion:** SHR are known to have larger strokes than non hypertensive strains. This study has shown that even very short duration vessel occlusions produce substantial infarction in SHR compared with non-hypertensive strains. This may be due to the limited collateral blood supply of the hypertensive animals.

POS-THU-272

SECRETED ALPHA-SYNUCLEIN IN EXOSOMES

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Alpha-synuclein is a key protein in Parkinson's disease (PD). Mutations in the gene are linked to heritable forms of the disease, and it is the main constituent of Lewy bodies, the intraneuronal inclusions which are characteristic of PD. α-synuclein is present in cerebrospinal fluid and blood plasma, and indirect evidence suggests that secretion by neurons may be a source of extracellular α-synuclein. The secretion mechanism is unknown. Sequence analysis and pharmacoligical studies suggest a non-classical, ER/Golgi-independent pathway. Other proteins linked to neurodegeneration and protein misfolding (prion protein and alzheimer precursor protein) have been identified in exosomes, a form of secreted vesicle released from multivesicular bodies (MVBs). We tested whether α-synuclein is present in exosomes derived from cell lines, including a model human dopaminergic neuron (SH-SY5Y). We found that α-synuclein colocalises inside the cell with markers of exosome biogenesis (eg Tsg-101 - a marker of late endosomes, MVBs and exosomes), but not with ER or Golgi markers. Exosomes purified from large volumes of conditioned media from SH-SY5Y, GT-17 (hypothalamic neurons) or HeLa cells were positive for exosome markers (Tsg-101 and Flotilin-1) and also contained an α-synuclein-immunoreactive band. This band had a higher molecular weight than recombinant or intracellular α-synuclein, and was positive for ubiquitin when reprobed. These data indicate that at least some proportion of secreted α-synuclein is post-translationally modified and found in exosomes. This may have implications for our understanding of the intracellular processing of α-synuclein, its role in vesicle trafficking and the potential for transsynaptic transmission of modified α-synuclein species.

POS-WED-273

MOTOR IMPAIRMENTS IN A MOUSE MODEL OF HUNTINGTON'S DISEASE WITH SELECTIVE ABLATION OF D1 RECEPTOR-EXPRESSING NEURONS IN THE STRIATUM

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Background: Clinical symptoms of Huntington's disease (HD) include hyperkinesia, gait and orofacial impairments, and cognitive deficits. Dopamine responsive medium spiny neurons in the striatum are preferentially lost in HD pathology. A transgenic mouse line with selective ablation of dopamine receptor D1R expressing striatal neurons was generated using the Cre-LoxP system under the control of DARPP-32 (dopamine and adenosine 3',5'-cyclic monophosphate (cAMP)-regulated phosphoprotein, 32kDa) promotor. **Purpose:** To understand which aspect of HD phenotype is due to the loss of D1R expressing striatal neurons, we have generated a model to specifically target the striatum. **Methods:** Mice (male WT n=10, MUT n=10; female WT n=10, MUT n=10) were assessed for abnormalities in locomotor activity, ethological assessment, hindlimb dystonia, rotarod test and gait analysis. Anxiety-like behaviours were assessed in the light/dark test, elevated plus maze and open field test. In addition, spatial working memory was tested in the spontaneous alternation test using Y-maze. **Results:** MUT showed reduced bodyweight, bradykinesia, impaired orofacial movements, impaired motor coordination, narrow base and smaller stepping gait, and a disturbed step sequence alternation pattern. MUT showed reduced anxiety-like behaviours, which may reflect a state of apathy that is found in HD patients, and unchanged spatial working memory. Behavioural differences existed between male and female mice. Interestingly, MUT did not display forelimb or hindlimb dystonia with tail suspension. **Conclusion:** These results demonstrate that D1R expressing striatal neurons are responsible for motor coordination, gait and orofacial movements. This is the first model to demonstrate the role played by D1R expressing striatal neurons in aspects of the HD phenotype.

POS-THU-274

ASTROCYTIC PATHOLOGY IN PARKINSONIAN SYNDROMES

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Purpose: To compare astrogliosis and its role in disease propagation in parkinsonian syndromes. Methods: Brain tissue donated for research to Brain Banks at POWMRI and Queen Square was analysed. Formalin-fixed, paraffin-embedded tissue (putamen, pons, substantia nigra) from 13 cases with Parkinson's disease (PD), 29 with multiple system atrophy (MSA), 34 with progressive supranuclear palsy (PSP) and 13 controls were cut at 5μm and stained for astrocytic (eg. GFAP, PACRG, parkin) and neuronal (eg. α-synuclein, tau) proteins using immunoperoxidase and double immunofluorescence. Differences from controls were quantified and correlated with pathological indices. Results: Protoplasmic but not fibrous astrocytes constituently expressed PACRG. In PD 40% of PACRG-positive astrocytes accumulated non-fibrillar α-synuclein that caused limited astrocytic reactivity and did not correlate with disease indices. In MSA only PACRG-negative fibrous astrocytes became reactive correlating with the degree of fibrillar α-synuclein-immunoreactive inclusions and disease stage. In PSP PACRG-positive astrocytes had marked reactivity expressing parkin and correlating with the degree of neuronal loss and phospho-tau-immunoreactive inclusions. A minority (20%) also contained phospho-tau. Conclusion: Marked but variable pathology was observed in different populations of astrocytes in these parkinsonian syndromes. In MSA and PSP astrocytic pathology directly related to disease progression.

POS-WED-275

LACK OF WIDESPREAD DEGENERATION AND α-SYNUCLEIN DEPOSITION IN MONKEYS WITH OVER 10 YEARS OF SEVERE MPTP PARKINSONISM

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Purpose: To determine whether aged monkeys with long-term parkinsonian symptoms following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration have similar pathology to patients with Parkinson's disease (PD). **Methods:** Formalin-fixed brains of two 14 year-old cynomolgus monkeys (*Macaca fascicularis*) were examined after 10 years of parkinsonism following 2 years of intermittent treatment with MPTP and compared with the brains of two 8 year-old control monkeys. Experiments were conducted under the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals by the United States National Institutes of Health and the European Union. Standard neuropathological protocols were used in addition to immunohistochemistry for tyrosine hydroxylase (TH). **Results:** Compared with controls, MPTP-primates had severe loss of TH-immunoreactive neurons and fibres in the substantia nigra (SN) and basal ganglia with sparing of nearby TH-immunoreactive neurons. Intraneuronal α-synuclein accumulation was observed in the few remaining SN neurons, not in the structural form of Lewy bodies but as a mass of aggregated particles. Extracellular Aβ aggregations were also found in the amygdala and temporal cortices. **Conclusions:** Despite the MPTP-primates having parkinsonism for a decade, no classic α-synuclein-immunopositive Lewy body inclusions were observed. This may suggest that either Lewy body-related pathologies are unique to humans, or that the neural environment associated with advanced age is necessary for Lewy-related pathologies.

POS-THU-276

PAX6 IN PARKINSON'S DISEASE

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The underlying pathology of PD is the degeneration of the substantia nigra (SN). Multiple hypotheses have been proposed to explain the vulnerability of the SN to PD, mainly focusing on the morphological properties of these cells (Richards et al. 1997), however understand the transcriptional response is also crucial (Sang et al. 2006). The *Pax* family of transcription factors are known to be critical during development in regionalisation, neurogenesis and differentiation of specific subpopulations of neurons. Furthermore they have been implicated in neural protection/regeneration in the adult. The *Pax* gene, *Pax6*, is expressed in the embryonic ventral midbrain and in subset of cells in the adult SN. **Purpose:** To determine whether *Pax6* expression in the SN is altered in an animal model of PD and if, *in vitro*, Pax6 has a neuroprotective effect. **Methods:** Experimental procedures were in accordance with Institutional Ethics Committees (Cambridge University, UK and University of Western Australia Ethics Committees), in all groups n=5. An animal model of PD was created and Pax6-immunopositive SN cells quantified and compared to normal adult SN. *In vitro*, the vulnerability to 6-OHDA was assessed (CellTitre-GLO Luminescent Cell Viability Assay) in differentiated PC12 cells and those that overexpressed *Pax6*. **Results:** The number of *Pax6* expressing SN cells is altered in an animal model of PD, while *in vitro* over-expression of *Pax6* in differentiated PC12 cells results in significantly less cells dying upon exposure to 6-OHDA. Conclusion: The reason for the continued expression of transcription factors in the adult brain is unclear. However their increased expression in injury/diseased states has lead to the hypothesis that the adult brain may be harnessing developmental events in an attempt to protect/repair itself.

POS-WED-277

CHARACTERISATION OF THE SUBSTANTIA NIGRA PARS COMPACTA IN THE ABSENCE OF NEURTURIN

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Purpose Neurturin, a member of the GDNF family, is a potent neurotrophic factor for dopaminergic cells and is currently being trialled as a therapy for Parkinson's disease patients. Previous analysis of neonatal neurturin knockout mice reported no gross abnormalities in the brain or any overt difference in tyrosine hydroxylase staining, however these analyses were qualitative. Here we show quantitative data on dopaminergic neurons of the substantia nigra in mice lacking neurturin. **Methods** Sections from 12-14 week old neurturin knockout mouse brains and their wildtype littermates (n=3) were sequentially labelled through the substantia nigra pars compacta with tyrosine hydroxylase (TH) and calbindin antibodies. Cell numbers were quantified using fluorescence stereology. The number of cell processes and cell sizes were estimated by visualisation of 3D data sets. **Results** No significant difference in the overall number of TH+ cells was found between knockout and wildtype mice. However, there was a significant increase in the number of TH+/calbindin+ cells in the knockout mice as compared with their wildtype littermates. **Conclusion** TH+/calbindin+ dopaminergic neurons of the substantia nigra have an increased survival potential in Parkinson's disease (Murase and McKay, 2006). Our results suggest a change in the composition of the nigral dopaminergic neurons in the absence of neurturin, namely, promoting a TH+/calbindin+ cell phenotype in the substantia nigra pars compacta.

POS-THU-278

PARKIN MEDIATED UBIQUITYLATION OF PARKIN CO-REGULATED GENE (PACRG) PROMOTES ITS RECRUITMENT TO AGGRESOMES

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Mutations in *parkin* are the most common genetic cause of early onset Parkinson's disease (EO-PD). Parkin functions in the ubiquitin-proteasomal system (UPS) as an E3 ubiquitin ligase and disruption of this pathway has been linked to the pathogenesis of PD. *PACRG* shares a bi-directional promoter with *parkin* and the two genes are co-regulated (West et al., 2003). The function of PACRG is unknown however we hypothesise that parkin and PACRG interact and function in a common pathway. **PURPOSE:** To confirm an interaction between parkin and PACRG and investigate its molecular consequences on PACRG localisation and function. **METHODS:** BE-(M17) neuroblastoma cells stably overexpressing PACRG or parkin were generated and co-immunoprecipitation (co-IP) analysis was performed. Truncated parkin constructs were generated to identify the parkin domain mediating the interaction. The functional consequences of the interaction were investigated by performing ubiquitylation assays, immunohistochemisty and microtubule stability studies. **RESULTS:** Co-IP studies demonstrated that parkin and PACRG interact in vivo, through the RING2 domain of parkin. Furthermore, parkin mediates the ubiquitylation of PACRG utilising both K48 and K63 ubiquitin linkages. The interaction and ubiquitylation of PACRG by parkin promoted the recruitment of PACRG to aggresomes. PACRG-positive aggresomes were observed in 82.0%±13 of M17 cells overexpressing parkin compared with 46.7%±11.8 of parental M17 cells (p=0.02, n=3) and were resistant to microtubule destabilisation by nocodazole. **CONCLUSION:** Our results suggest that parkin and PACRG function in a common pathway, potentially involving microtubule-mediated aggresome formation. Aggresomes may represent a cellular defence mechanism against the toxic effects of misfolded proteins and disruption of this process may contribute to the pathogenesis of PD.

POS-WED-279

TORSINA, A PROTEIN ASSOCIATED WITH EARLY ONSET DYSTONIA, INTERACTS WITH COLLAPSING RESPONSE MEDIATOR PROTEIN

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The dystonias represent a heterogeneous group of neurological disorders characterised by involuntary muscle contraction and twisting, repetitive movements. Mutation of the torsinA gene (DYT1) causes early-onset general torsion dystonia, the most common and severe form of dystonia. While previous studies have indentified several torsinA interacting proteins and suggested potential roles for torsinA in nuclear membrane morphology and protein transport, the function of torsinA is currently unknown. Purpose: We have searched for novel binding partners of torsinA to determine the mechanism by which mutation of torsinA might produce a neurological phenotype and to investigate the molecular pathways disrupted in dystonia.Methods: Potential interactors were identified using an unbiased proteomics approach. TorsinA was immunoprecipitated from human cortex and associated proteins were identified by LC-MSMS. TorsinA-interacting proteins were further confirmed by co-immunoprecipitation and localisation studies. Results: We have identified the collapsin response mediator protein (CRMP2) as a torsinA interacting protein. The interaction was confirmed by in vivo co-immunoprecipitation analysis of endogenous and exogenous CRMP2 in both HEK-293 and human neuroblastoma cells (BE-M17) using two independent torsinA antibodies. Futhermore, we examined the effect of the dystonia specific mutation (ΔE302/303) and mutations within functional domains of torsinA on the interaction between torsinA and CRMP2. Conclusion: We have identified CRMP2 as a novel protein that interacts with torsinA. CRMP2 has been associated with neurological disorders such as Alzheimers disease is thought to be involved in neuronal microtubule assembly and axon outgrowth. Our results suggest that disruption of the interaction between torsinA and CRMP2 may contribute to the development of dystonia.

POS-THU-280

UNLOCKING THE SECRETS OF FAMILIAL EPILEPSY: A WHOLE GENOME ANALYSIS

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In the past ten years a number of familial epilepsies have been associated with single ion channel gene mutations. In a bid to understand fundamentals of disease genesis in familial epilepsy, we generated gene targeted knock-in mouse models harbouring human ion channel mutations found in families with epilepsy. Two such models have been utilised in this study: the R43Q mutation, located in the γ2-subunit of the $GABA_A$ receptor and C121W mutation, within the β-subunit of the voltage-gated sodium channel. Aim: To identify genes and biological pathways which are modulated in the context of the whole brain in two clinically relevant models. Method: Microarray technology was used to identify differentially expressed genes in wild type and knock-in mice from at least 3 individual male brains for two models and at two ages: R43Q heterozygote mice (P14 and P40). C121W heterozygote (P14 and P40) and homozygote mice (examined at P14 only due to premature death at P21); wild type litter mates (n=3) were controls for each group. Total RNA was isolated and hybridised to the Affymetrix mouse all exon array. Differential expression was analysed using gene summary data by Partek Genomics Suite (Version 6.3) and GOminer. Results: At P14 in the C121W model, a number of cell adhesion molecules is increased in knock-in mice compared to controls ($p<0.01$). In R43Q model a 2 way ANOVA identified ion channels exhibiting an age-genotype interaction ($p<0.001$). Conclusion: Genes already implicated by their association with ion channel function or epilepsy, were found differentially expressed in these single ion channel mutation models. In addition, our findings have revealed pathways that may underlie seizure genesis with implications for diagnosis and therapy.

POS-WED-281

CELLULAR MECHANISM OF HYPEREXCITABILITY IN THE β1 (C121W) MOUSE MODEL OF HUMAN EPILEPSY

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SCN1B encodes a sodium channel accessory subunit. A cysteine to tryptophane (C121W) mutation in *SCN1B* has been associated with the human epilepsy syndrome, GEFS+ (generalized epilepsy with febrile seizures plus). In vitro experiments have detected a range of kinetic and cell biological defects. In vivo analysis has been limited, and thus there is little known about the impact of this mutation on neuronal function. We have created a knockin mouse carrying the β1 (C121W) mutation. Heterozygous mice recapitulate the human phenotype, showing increased sensitivity to thermal seizures. We combine this analysis with a morphological study of the distribution of the wild type and mutant β1 forms using virus encoded green fluorescent protein-tagged subunits delivered by in vivo injection. Furthermore, whole cell current clamp recordings in hippocampal brain slices demonstrate action potential shortening, consistent with a pro-epileptic effect of β1 (C121W) in vivo. Our results relate disruption of intracellular β1 targeting with a hyperexcitable electrophysiological phenotype and thus, reveal a cellular pathogenic mechanism for seizure generation in vivo.

POS-THU-282

EPILEPSY CAUSING ION CHANNEL MUTATIONS IN THE DENTATE GYRUS

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In recent years hundreds of epilepsy mutations in sodium channels have been identified. These mutations have various effects including loss of expression, inappropriate trafficking and changes in gating. These all ultimately lead to electrophysiological changes which will alter how the neurons integrate input and ultimately how the network behaves. Typically, mutations have several effects simultaneously, some of which increase neuron excitability and some of which will decrease excitability. Predicting the combined effect of several changes, and how these changes alter network excitability is difficult. In this study, we used computer simulation of a realistic model of the dentate gyrus (DG) to understand the link between molecular lesion, cellular phenotype and network excitability. Some sodium channel mutations have been linked to temporal lobe epilepsy and changes in excitability of the DG cause, or are caused by, temporal lobe seizures. The network model contained morphologically and electrophysiologically realistic models of granule, mossy, HIPP and basket cells with realistic patterns of connections. We performed a sensitivity analysis by altering sodium channel gating properties in ways typically seen in mutated versions. The network was stimulated with constant frequency, random input from the perforant path. In the control case, the network showed moderate accommodation in response to long duration inputs. Left shifting the voltage dependence of activation reduced the accommodation and a right shift increased accommodation. By contrast, altering activation rates and inactivation had little effect. In all cases the network returned to rest on cessation of input and no other stables states were found. In conclusion, the DG is unlikely to be the focus of seizures caused by ion channel mutations without additional structural changes. However, mutations will effect how much activity flows into deeper structures and this together with changes in these structures may explain hippocampal based seizures.

POS-WED-283

ENVIRONMENTAL ENRICHMENT DELAYS THE ONSET OF LIMBIC EPILEPSY AND IMPROVES ANXIETY-LIKE AND NEUROCOGNITIVE BEHAVIOURS

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Purpose: Temporal lobe epilepsy (TLE) is the most common adult epilepsy syndrome although one-third of patients remain refractory to current pharmacological treatment. In addition, TLE is commonly accompanied by neuropsychiatric and neurocognitive comorbidities including anxiety, depression and learning deficits. Given recent animal studies highlighting enhanced seizure susceptibility following stress, it was of interest to examine the neuroprotective capacity of 'positive experiences' created using environmental enrichment (EE). **Methods**: Male Wistar rats were randomly allocated into one of two housing conditions at weaning: EE (large plastic cages containing running wheels, assorted bedding and toys) or Impoverished Housing (IH; standard laboratory cages with sawdust bedding only). Bipolar electrode implantation into the left amygdala was performed at P63, followed by rapid amygdala kindling at P70. The development of epilepsy was scored using the Racine scale of behavioural seizures with rats deemed fully-kindled after five class V seizures. The Elevated Plus Maze (EPM) and Morris Water Maze (MWM) behavioural tests were conducted to assess anxiety and spatial learning, respectively. **Results**: EE delayed the time-course of seizure progression, with enriched rats (n=16) requiring a significantly greater number of kindling stimulations to elicit the first class V seizure and to reach a fully-kindled state compared to IH rats (n=13; $p<0.05$). EE also reduced anxiety-like behaviour in the EPM (EE: n=27, IH: n=23; $p<0.05$) and facilitated superior performance in the MWM (EE: n=16, IH: n=13; $p<0.05$). **Conclusion**: Our data demonstrates a beneficial effect of prolonged EE on vulnerability to limbic epilepsy, co-morbid anxiety and neurocognitive function.

POS-THU-284

AP-1 INHIBITORY PEPTIDES REDUCE NEURONAL DEATH IN AN *IN VITRO* EPILEPSY MODEL

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Purpose: Epilepsy is the second most common neurologic disorder causing progressive neuronal cell loss with subsequent seizures. This is an area of particular clinical relevance as currently there are no therapies available to prevent neuronal cell death following epileptic seizures. In order to study cell death processes following seizure activity, epilepsy has been modeled in small animals, such as rats and mice, as well as in neuronal cultures by kainic acid exposure. **Methods:** Five peptides that we have demonstrated to inhibit AP-1 activation (eg c-Jun/c-Fos activation) were tested for their ability to prevent neuronal death in an *in vitro* kainic acid model. Additionally, the ability of the AP-1 inhibitory peptides to reduce alpha-fodrin cleavage, a marker of caspase induced neuronal death, was assessed following kainic acid exposure (n=3). **Results:** Primary cortical neuronal cultures (n=4) treated with either of the five AP-1 inhibitory peptides reduced neuronal cell death significantly ($p<0.05$) in a dose dependent manner. All five peptides reduced caspase induced alpha fodrin cleavage when compared to untreated neuronal cultures in the kainic acid model. **Conclusion:** These inhibitory peptides are efficacious at inhibiting neuronal death following kainic acid exposure. They are currently being truncated and further assessed in the epilepsy model to ascertain the shortest amino acid sequence that can maintain neuroprotective efficacy.

POS-WED-285

INCREASED EXPRESSION OF FAS RECEPTOR AND LIGAND IN PREFRONTAL GREY MATTER FROM PATIENTS WITH SCHIZOPHRENIA

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Purpose: The observation that cancer occurrence in patients with schizophrenia is incongruent with patient's exposure to cancer risk factors has led to the hypothesis that proneness towards apoptosis plays a role in the development of schizophrenia whilst being protective against cancer occurrence. The observed neuropathology of schizophrenia includes a marked reduction in dendritic spines in the absence of large scale neuronal cell death and is consistent with apoptotic pathways having a sublethal but still deleterious role in disease development. Examination of microarray findings of the Stanley Medical Research Institute collection of postmortem brain tissue revealed increased prefrontal expression of molecules in tumour necrosis factor death receptor pathways in people with schizophrenia, including the putative FAS receptor ligand, TNFSF13 and the FAS receptor. The current study sought to replicate these findings. **Methods:** Total RNA was isolated from the dorsolateral prefrontal cortex of patients (n=37) and controls (n=37) matched on age, pH, RNA integrity, and postmortem interval. Expression of target genes was measured by quantitative real time RT-PCR. **Results:** As predicted, there was a significant increased expression of the TNFSF13 mRNA in patients relative to controls ($p<0.05$, one-tailed t-test). The expression of TNFSF13 correlated negatively with pH. There was a trend for an increased expression of FAS receptor mRNA ($p<0.1$, one-tailed t-test). **Conclusion:** This study replicates previous microarray results in an independent cohort. The increased expression of FAS receptor pathway molecules in schizophrenia is consistent with increased apoptotic drive within grey matter cells, and the correlation of TNFSF13 expression with tissue pH suggests this may lead to an oxidative environment.

POS-THU-286

PRESYNAPTIC MARKER mRNAS ARE ALTERED IN THE DORSOLATERAL PREFRONTAL CORTEX IN SCHIZOPHRENIA

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Purpose: A dysregulation in synaptic transmission may contribute to altered cognitive processes in schizophrenia. Several pre-synaptic markers have been examined in schizophrenia, however changes in expression levels reported in the disease remain inconsistent. We have examined the relative expression of the synaptic protein synaptobrevin/vesicle-associated membrane protein (VAMP, a member of the SNARE complex) and the presynaptic plasticity-associated protein growth associated protein-43 (GAP-43), in addition to proteins enriched in glutamatergic terminals (vesicular glutamate transporter (VGLUT) and complexin 2), and GABAergic terminals (vesicular GABA transporter (VGAT) and complexin 1) in a large cohort of schizophrenia/schizoaffective patients. **Methods:** Total RNA was isolated from the dorsolateral prefrontal cortex of patients (n=37) and controls (n=37) matched on age, pH, RNA integrity number, and post mortem interval. Expression of target genes was measured by quantitative real time RT-PCR. **Results:** Preliminary non-normalised data show a 15.8%, 11.8% and 11.0% reduction in average GAP-43 ($p=0.0079$), complexin 1 ($p=0.049$) and VAMP ($p=0.065$) mRNA expression, respectively in schizophrenia cases compared to matched controls. Complexin 2, VGLUT and VGAT mRNA expression were not altered significantly ($p>0.05$). **Conclusion:** A reduction in VAMP mRNA may implicate a general deficit in synaptic neurotransmission, and concomitant reductions in GAP-43 and complexin 1 suggest that this deficiency may relate to reduced plasticity of excitatory terminals as well as reduced integrity of inhibitory terminals. However, not all presynaptic markers are altered, suggesting that the overall density and structure of terminals may be intact in schizophrenia, while certain functions and/or subsets of terminals may be particularly vulnerable.

POS-WED-287

MARKERS OF INTERNEURONS IN SCHIZOPHRENIA

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Introduction: Schizophrenia is a complex neuropsychiatric disorder involving perceptual, behavioural and cognitive abnormalities. Recent studies have demonstrated deficient cortical inhibition among patients with schizophrenia which have implicated a possible role for gama-amniobutyric acid (GABA) neurons in this disorder. Several independent molecular and neuroanatomic studies have now provided evidence that bolster the argument that GABAergic neuronal dysfunction is mechanistically involved in schizophrenia. **Purpose:** In this study we assessed altered expression of interneuron markers, somatostatin, parvalbumin, calretinin, calbindin, cholecystokinin and neuropeptide Y in schizophrenia. **Method:** We used real time quantitative RT-PCR to assess the expression levels of the various interneuron markers in a cohort of 37 schizophrenia cases and matched 37 control samples from the New South Wales Tissue Resource Centre. **Results:** We found a significant ($p = 0.002$) reduction by 27.5% in the expression of somatostatin after normalising with the geometric mean of two housekeeper genes ubiquitin C and hydroxymethylbilane synthase in schizophrenia cases. We also found a 10% reduction in the expression of parvalbumin ($p = 0.044$) in schizophrenia cases. **Conclusion:** Our findings provides further support for abnormal inhibitory function of somatostatin expressing interneurons in schizophrenia and corroborate recent reports of reduced markers of specific interneurons in schizophrenia.

POS-THU-288

LEARNING AND MEMORY IN RATS WITH SEROTONIN DEPLETION OF THE DORSAL AND VENTRAL HIPPOCAMPUS

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Purpose: The neuropathology of schizophrenia includes abnormalities in serotonergic transmission. Rats with serotonin depletion in the dorsal hippocampus (DH), but not ventral hippocampus (VH), display enhanced locomotor hyperactivity following treatment with phencyclidine and disruption of prepulse inhibition — behavioural models of aspects of schizophrenia. This study aims to investigate the effect of DH and VH serotonin depletion on learning and memory, to model the cognitive deficits in schizophrenia. Methods: Male Sprague-Dawley rats (250-300g) were isoflurane-anaesthetised and stereotaxically microinjected with the serotonergic neurotoxin, 5,7 dihydroxytryptamine, into either the DH or VH ($n\geq9$/group). Results: The first cohort was tested in the Y-maze and Morris water maze to investigate spatial learning. After 1 and 2 hr intervals in the Y-maze, all rats showed intact short-term spatial memory as measured by an increased number of entries and duration of time spent in the novel arm. In addition, all groups showed intact long-term spatial memory as measured by the Morris water maze. A second cohort of rats was tested for working memory in the Alternating T-maze. There were no working memory differences between groups in the T-maze cohort following the interposition of 30 and 60 sec delays. VH-lesioned rats, however, took significantly longer than sham-operated controls to learn the alternating task. ELISA showed marked depletion in the DH or VH in the respective lesion groups. Conclusion: These findings are unexpected, given the role of the DH in spatial learning and memory, and the salient behavioural change that DH serotonin depletion induces in other models of schizophrenia. Our results suggest a functional redundancy of serotonin in the hippocampus on mechanisms of learning and memory.

POS-WED-289

INCREASED BDNF EXPRESSION IN THE HIPPOCAMPUS OF 5-HT$_{2C}$ RECEPTOR KNOCKOUT MICE

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Purpose: Brain derived neurotrophic factor (BDNF) stimulates growth and differentiation of neurons and synapses and is involved in memory and neuroplasticity. Altered expression of brain-derived neurotrophic factor (BDNF) in the brain has been implicated in schizophrenia and mood disorders, such as anxiety and depression. Several studies have suggested a close interaction between BDNF and serotonin (5-HT), however no previous studies have specifically reported a relationship between BDNF and the 5-HT$_{2C}$ receptor. The aim of this study was to investigate this interaction by determining BDNF expression in 5-HT$_{2C}$ receptor knockout mice. Methods: Brain tissue was obtained from male 5-HT$_{2C}$ receptor knockout mice which were bred and genotyped at the Mental Health Research Institute. BDNF protein expression was assessed by Western blot analysis of homogenates of hippocampus, frontal cortex and striatum. Results: There was a significant 2.2-fold increase in the expression of the mature form of BDNF in the hippocampus of 5-HT$_{2C}$ receptor knockout mice when compared to wildtype littermate controls ($p=0.005$, $n=13$). No differences were found in this brain region in the expression of the immature, 37 kDa, 25kDa, 19 kDa or 16kDa pro-BDNF products. This was a region-specific effect as all BDNF expression levels were unchanged in the frontal cortex and striatum. In addition, no differences were found in the expression of the BDNF receptor, TrkB, in hippocampal and frontal cortex homogenates. Conclusion: Loss of 5-HT$_{2C}$ receptor expression leads to a marked and selective increase in the expression of the mature form of BDNF in the hippocampus. These increased BDNF levels may have behavioural consequences, such as on learning and memory.

POS-THU-290

PREPULSE INHIBITION OF STARTLE IN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) HETEROZYGOUS MICE

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Purpose: BDNF expression is reduced in the prefrontal cortex of patients with schizophrenia. Prepulse inhibition (PPI) is a measure of sensory gating which is deficient in schizophrenia. We therefore assessed PPI and its modulation by dopaminergic and serotonergic drugs in mice heterozygous for a mutation in the BDNF gene (BDNF Het). Methods: BDNF levels were verified by Western Blot analysis. PPI of acoustic startle was measured at two different inter-stimulus intervals (ISI, 30 msec and 100 msec) and four prepulse intensities (2, 4, 8 and 16 dB over baseline) using automated startle boxes. Mice (n=10-12) were 10-16 weeks of age. Results: As expected, BDNF protein levels were significantly reduced by approximately 50% in the hippocampus of mutant mice. However, baseline PPI and startle were not different between BDNF Het and wildtype controls. Treatment with the dopaminergic receptor agonist, apomorphine (5 mg/kg), the dopamine releaser, amphetamine (5 mg/kg), or the glutamate NMDA receptor antagonist, MK-801 (0.25 mg/kg), caused disruption of PPI but there was no difference between the genotypes. Treatment with the serotonin uptake ligands, fluoxetine (10 mg/kg) or fenfluramine (10 mg/kg), increased PPI to a similar extent in BDNF Het and wildtype mice. Treatment with MDMA (Ecstacy) caused disruption of PPI at the 30 msec ISI and this effect was greater in BDNF Het than in wildtype controls. Conclusion: These studies show that, unlike in patients with schizophrenia, in mice significant reduction of BDNF expression is not associated with disrupted PPI at baseline. With the exception of MDMA, BDNF Het mice were also not differentially sensitive to the action of dopaminergic and serotonergic drugs on PPI.

POS-WED-291

COMBINED TARGETING OF MGLU5, A2A AND CB1 RECEPTORS TO REGULATE ALCOHOL SELF-ADMINISTRATION AND CUE-CONDITIONED ALCOHOL SEEKING IN RATS

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Purpose: We have recently demonstrated a synergistic interaction between the mGlu5 receptor antagonist, MTEP, and A2A receptor antagonist SCH 58261 to reduce operant responding for alcohol in iP rats (1). To further examine possible receptor-receptor interactions, the CB1 receptor antagonist SR-141716A was co-administered with MTEP or SCH 58261 in an operant paradigm with iP rats [n=10] self-administering 10% v/v ethanol solution from the active lever and water from the inactive lever. Cue conditioned alcohol seeking was also examined. Results: Administered alone, SR141716A significantly reduced ethanol-self administration at doses of 0.3mg/kg and greater. When co-administered with MTEP or SCH 58261 at either sub-threshold or threshold doses of each antagonist, SR-141716A did not produce a greater-than-additive reduction in ethanol-self-administration. iP rats show robust alcohol-seeking following incubation of craving. MTEP at 1.0mg/kg co-administered with SR-141716A at 0.3mg/kg i.p. significantly reduced cue-conditioned alcohol seeking behaviour (-84.2%, $p<0.001$) compared to vehicle-treated iP rats. In contrast, SCH 58261 at 2mg/kg co-administered with SR-141716A at 0.3mg/kg i.p. did not reduce lever-pressing during cue-conditioned alcohol seeking behaviour (-3.5%, $p=1.0$). Conclusion: Although CB1 receptors are co-localized with mGlu5 and A2A receptors in many areas implicated within addiction, CB1 receptors do not appear to synergistically interact with A2A or mGlu5 receptors to regulate ethanol self-administration. Additionally, prevention of cue-conditioned alcohol seeking seems to be primarily mediated through antagonism of combinations including the mGlu5 receptor. Adams et al. (2008) Int J Neurophsychopharm.

POS-THU-292

CHARACTERIZATION OF MICE WITH A TARGETED DELETION OF CREB IN STRIATAL MEDIUM SPINY NEURONS

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Purpose: The transcription factor cAMP response element binding protein (CREB) has been implicated in addiction because its activation is regulated in brain reward regions in response to drug administration. The aims of the present study were to characterize mice with a deletion of CREB in striatal medium spiny neurons with respect to locomotor sensitization to cocaine. The deletion of CREB from the striatum was confirmed, and the expression of related transcription factors was also assessed. **Methods:** Mutant mice were generated using the "cre/lox" recombination system where DARPP-32 was used to drive the expression of cre-recombinase. Control littermates and CREB$^{DARPP\text{-}32Cre/loxlox}$ mice (n=20 and n=14 respectively) were pretreated with either cocaine (20mg/kg, i.p.) or saline (10ml/kg, i.p.) for 5 days before being monitored in locomotor chambers. Following 7 days of abstinence the mice were administered a challenge dose of cocaine (10mg/kg, i.p.) and their locomotor activity assessed. Immunohistochemistry was used to confirm the CREB deletion and quantitative RT-PCR was used to examine the expression of CREB and related genes in naïve mice. **Results:** CREB$^{DARPP\text{-}32Cre/loxlox}$ mice displayed enhanced locomotor activation compared to wild types upon acute cocaine exposure. Following chronic cocaine treatment, CREB$^{DARPP\text{-}32Cre/loxlox}$ mice exhibited attenuated development of sensitization, however the expression of sensitization was enhanced following a 7 day withdrawal period. Immunohistochemistry confirmed the deletion of CREB from the striatum of CREB$^{DARPP\text{-}32Cre/loxlox}$ mice, and quantitative RT-PCR data revealed a compensatory increase in the striatal expression of the related factor CREM as a result of the deletion. **Conclusions:** This suggests that striatal CREB may be involved in the development but not the expression of locomotor sensitization to cocaine.

POS-WED-293

SECONDARY DEATH OF RETINAL GANGLION CELLS, AND THE EFFECTS OF A CALCIUM CHANNEL BLOCKER, LOMERIZINE

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Purpose: Following partial optic nerve injury, intact retinal ganglion cells (RGCs) undergo secondary death, but it is unclear which cell death pathways are involved. Also, although the calcium channel blocker lomerizine reduces RGC death following partial optic nerve injury, it is unknown whether this drug alleviates apoptosis, necrosis or both. Here we determined whether secondary RGC death was characterized by caspase-dependent apoptosis or necrosis and the extent to which lomerizine rescued each. **Methods:** The dorsal optic nerve was transected in adult PVG rat and the site of primary RGC death determined using retrograde DiI tracing (n=2). Overall RGC loss was examined using βIII-tubulin immunohistochemistry at 2 and 3 weeks (n=9). Intact RGCs undergoing secondary death were identified by retrograde tracing with fluorogold injected into the superior colliculus 11 or 18 days after injury and quantified in retinal wholemounts using anti-cleaved caspase-3 immunohistochemistry for caspase-dependent apoptosis and nucleic acid stain sytox green for necrosis (n=40). **Results:** Ventral retina was identified as the site of secondary cell death while central and dorsal retina were defined as sites of both primary and secondary RGC death. Overall RGC loss occurred by 2 weeks in central and ventral retina ($p<0.05$), and by 3 weeks dorsally ($p<0.05$). Secondary RGC loss was mainly necrotic, with some caspase-dependent apoptosis. Lomerizine reduced secondary necrosis at 2 weeks and secondary caspase-dependent apoptosis at 3 weeks. **Conclusion:** Lomerizine's differential effects on necrosis and apoptosis with time, and its inability to completely prevent secondary death, suggest that full protection will require combinatorial treatments.

POS-THU-294

LOCALISATION OF THE LOW AFFINITY CATECHOLAMINE BINDING SITE IN TYROSINE HYDROXYLASE

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PURPOSE: Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of the catecholamines dopamine, noradrenaline and adrenaline. TH controls the rate of production of catecholamines in cells. Short-term control of TH activity is achieved through a combination of feedback inhibition by the catecholamines and reactivation by phosphorylation. Catecholamines bind with high affinity and essentially irreversibly to TH to inhibit the enzyme. Phosphorylation of TH induces dissociation of bound catecholamine, thereby increasing enzyme activity. We have identified a second catecholamine inhibitory site which is readily reversible and functions independently of the phosphorylation state of the enzyme. This site therefore controls the level of cytosolic catecholamines under both basal and stimulated conditions. The aim of this work was to use in vitro mutagenesis techniques to determine the position of the low affinity catecholamine binding site in TH. **METHODS:** The crystal structure of TH was used to identify residues in the active site of TH that could have a role in the low affinity site. The dopamine dependent inhibition of TH activity in wild-type and mutant TH molecules was measured. **RESULTS:** The EC_{50} for the dopamine inhibition in the Tyr371Phe TH mutant was 70 fold higher than the EC_{50} for the dopamine inhibition in wild-type TH ($p<0.005$, n=3). The EC_{50} for the dopamine inhibition in the Leu371Ala TH mutant was 2.5 fold higher than the EC_{50} for the dopamine inhibition in wild-type TH ($p<0.0005$, n=3). **CONCLUSIONS:** The results from this work indicate that the low affinity catecholamine binding site is localised to the active site of TH.

POS-WED-295

THE LOW AFFINITY DOPAMINE BINDING SITE REGULATES TYROSINE HYDROXYLASE ACTIVITY IN SITU: IMPLICATIONS FOR THE REGULATION OF CYTOSOLIC CATECHOLAMINE LEVELS

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Purpose: Tyrosine hydroxylase (TH) is regulated by phosphorylation of 3 key serine residues and by feedback inhibition by the catecholamines. We have recently demonstrated that dopamine inhibits TH by two distinct mechanisms. It is well-known that dopamine can bind with high affinity to TH, and once bound in this way is only dissociated following phosphorylation of Ser40. In addition, dopamine can bind to TH in a low-affinity, readily-dissociable manner. This form of inhibition is not phosphorylation-dependent in vitro. The K_D of dopamine binding to this low affinity site is similar to the concentration of cytosolic catecholamines, suggesting that dopamine inhibition via this site may be dependent on cytosolic catecholamine levels. **Methods:** PC12 cells were incubated with 20μM L-DOPA for 24 hours, and in situ TH activity and phosphorylation was measured. **Results:** L-DOPA had no effect on cell viability. L-DOPA significantly inhibited in situ TH activity to 25% of control levels ($p<0.001$, n=5). Stimulation of PC12 cells for 10 minutes with 1μM forskolin increased the phosphorylation of Ser40, and significantly increased TH activity ($p<0.05$, n=5). Pre-incubation of cells with L-DOPA significantly inhibited this forskolin-induced increase in activity ($p<0.05$, n=5). L-DOPA did not affect the phosphorylation of Ser40 or total TH protein levels. **Conclusion:** The low affinity dopamine binding site is able to inhibit TH activity in situ by responding to increases in cytosolic catecholamine levels under both basal and stimulated conditions. Thus, the low affinity site may act to maintain an equilibrium of cytosolic catecholamine levels, preventing the potentially harmful accumulation of cytosolic catecholamines.

POS-THU-296

ADVANCED PATERNAL AGE IS ASSOCIATED WITH ENLARGEMENT OF THE ROSTRAL CORTEX AND INCREASED ANXIETY-RELATED BEHAVIOUR IN C57BL/6J MICE

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Purpose: Advanced paternal age (APA) is associated with an increased risk of neurodevelopmental disorders in adult offspring, including schizophrenia and autism. We have shown that APA in mice is associated with changes in the size of the lateral ventricles and cortical width in adult male offspring. The aim of the present study was to investigate changes in cortical volume of adult APA mice in detail, using both magnetic resonance imaging (MRI) and stereological techniques and to examine the consequences on a range of behavioural domains. **Methods:** The male offspring of 12-18 month-old (APA) and 4 month-old (Control) C57Bl/6J sires underwent a behavioural test battery comprising tests for locomotion, spatial memory, anxiety and exploration. The mice were then killed at 12 weeks and their brains imaged ex vivo using the 16.4T animal MRI facility (Bruker BioSpin; Centre for Magnetic Resonance, University of Queensland). Following imaging, 50μm coronal sections were cut on a freezing microtome and stained with 0.1% cresyl violet in acetic buffer. Cortical neuron number was estimated by the optical fractionator method using StereoInvestigator software (MBF Biosciences). **Results:** Total cortical volume was unchanged by APA. However, the portion of the cortex rostral to the lateral ventricles was 16% larger in male APA mice, relative to control males ($p=.02$). Stereological analysis revealed no significant effect of APA on cell number or density in the rostral cortical region. These changes were not associated with changes in the behavioural domains of locomotion, spatial memory or exploration. However, compared to control males, male APA mice demonstrated increased anxiety-related behaviour on the elevated plus-maze (EPM; $p=.04$). **Conclusion:** We show that APA mice had increased cortical volume rostral to the lateral ventricles and increased anxiety-related behaviour. The absence of a change in cell number or density in the rostral cortical region may indicate an increase in cell size in the rostral cortex or an as yet unidentified alteration in dendritic and glial processes. Since cortical growth is often disrupted in cases of autism, these results provide some support that the APA model reflects aspects of this disorder.

POS-WED-297

INVESTIGATION INTO THE INTERACTION BETWEEN TISSUE-TYPE PLASMINOGEN ACTIVATOR AND DEAD NEURONAL CELLS

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Purpose: Tissue-type plasminogen activator (t-PA) is a protease appreciated for its role in intravascular fibrinolysis. For fibrinolysis to occur, t-PA and plasminogen (plg) must co-localise on fibrin, after which t-PA cleaves plasminogen to plasmin. Hence, fibrin acts as a co-factor for t-PA-mediated plasmin formation in the blood. t-PA-mediated plasmin formation is not only involved in haemostasis, but also in brain function and dysfunction. Unlike the blood, however, the brain is devoid of fibrin. Accordingly, this study addresses what non-fibrin co-factors drive t-PA-mediated plasmin formation in the brain under injury. Methods: t-PA- and plg-binding to injured cells was visualized by immunocytochemistry and western blot. Plasmin activity was measured by chromogenic S2251 assay. Flow cytometry was used to monitor cellular degradation. Results: Current results indicate that t-PA binds to neurons that have been injured either *in vitro* or *in vivo*. This interaction is specific and primarily relies on the so-called Finger, EGF and/or K1 domains of t-PA. In addition, our data suggests that t-PA retains its proteolytic activity when bound to injured neurons (n=3). Plg also binds to dead neurons. Unlike t-PA-binding, plg-binding to dead neurons entirely relies upon lysine residues (n=3). Moreover, we demonstrate that dead neurons (and dead non-neuronal cells) facilitate t-PA-mediated plasmin formation (n=3). In addition we show that this co-factor activity leads to plasmin-mediated proteolytic degradation of dead cells (n=4). Lastly, our data indicates that t-PA-binding correlates with the formation of protein aggregates within injured neurons. Currently we are examining the affect of t-PA on the phagocytosis of dead cells and furthermore the affect of plasmin-mediated dead cell degradation on phagocytosis. Conclusion: The aggregation of proteins during cell injury acts of a co-factor for t-PA-mediated plasmin formation and subsequent dead cell degradation.

POS-WED-299

PROGRAMMED CELL DEATH IN CEREBELLAR GRANULE CELLS INDUCED BY INHIBITION OF MITOCHONDRIAL RESPIRATORY CHAIN COMPLEXES

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Purpose: Various forms of programmed cell death (PCD) including apoptosis, autophagy and endoplasmic reticulum (ER) stress may contribute to neurodegeneration. Differential recruitment of these forms of PCD produces the resultant signature of neuronal injury. The aim of this study was to investigate how inhibition of mitochondrial respiratory chain complexes produced PCD and how the cellular context determines the recruitment of injury cascades. **Methods:** Primary cultures of cerebellar granule cells (CGCs; d7 C57Bl6 mice) were exposed for 1-24 hr on 7 div to insults targeting mitochondrial respiratory chain complexes I-IV (rotenone, 3-nitropropionic acid, antimycin A and KCN, respectively). Injury was analysed by a MTT cell viability assay (index mitochondrial activity) and cellular morphology was examined by phase contrast microscopy. Apoptotic-like injury was induced by parallel use staurosporine. Progression of PCD was analysed by labelling CGCs with Annexin V and PI. **Results:** Each respiratory chain inhibitor produced a concentration-dependent profile of injury - IC_{50} values (n = 5-7 independent experiments) as follows: rotenone 0.35 µM, 3-nitropropionic acid 13 µM, antimycin A 23 nM and KCN 7.9 µM. Phase contrast images showed breakdown of neuritic networks after drug treatment. Thus inhibition of mitochondrial respiratory chain complexes induced a time-dependent injury of CGCs across an apoptotic-necrotic continuum as shown by changes in all parameters measured. **Conclusion:** These patterns of injury were suggestive of PCD occurring across a time- and insult-dependent continuum. Ongoing work focuses on how components of autophagy and ER stress might contribute to the mechanisms of PCD described herein.

POS-THU-298

PHENOTYPING NEURONAL INJURY: INSULT-DEPENDENT CROSSTALK BETWEEN CASPASE-DEPENDENT/-INDEPENDENT CELL DEATH PATHWAYS

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Programmed cell death (PCD) occurs in various neuropathologies and can be triggered by mitochondrial dysfunction through stressors effecting redistribution of apoptogenic proteins. Neurons manifest canonical death processes, although “classical” apoptotic death involving the “intrinsic” pathway and caspase activation is not routinely observed. Altered Ca2+ can induce opening of the outer mitochondrial membrane (OMM) and activate enzymes, such as calpains, which share specificity for caspase substrates and interact with apoptogenic proteins to induce caspase-independent PCD. We determined whether an interactive model of caspase-dependent/-independent injury with system-dependent crosstalk applied to PCD. Using primary neuronal cultures (excitatory glutamatergic cerebellar granule cells (7d Swiss mice) and inhibitory striatal GABAergic neurons (E18 C57Bl6 mice)) we determined the progression of PCD, involvement of proapoptotic mitochondrial signaling and patterns of protease activation. Concentrations of stressors, including the apoptotic-like inducer staurosporine, were adjusted to produce time-dependent Annexin V labeling. Immunocytochemical analyses of the redistribution of apoptogenic proteins, including cytochrome c and apoptosis-inducing factor (AIF), revealed all insults (oxidative stressors, excitotoxicity, trophic-factor deprivation, inhibitors of complexes I/II) elicited redistribution of AIF, although this response was insult-dependent. Variable activation of caspase-3 and calpain was found. GABAergic striatal neurons exhibited more prominent caspase-independent PCD than glutamatergic neurons. These data demonstrated the hierarchical nature of mitochondrial participation in neuronal injury after permeabilization of the OMM. By definition of the “death” machinery we developed canonical models of neuronal PCD.

POS-THU-300

SIMVASTATIN DECREASES GLUTATHIONE CONTENT IN ASTROCYTES: IMPLICATIONS FOR TREATMENT OF NEURODEGENERATIVE DISEASES

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Purpose: Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), used for the treatment of hypercholesterolemia, are reported to have antioxidant properties and may therefore, have value in the treatment of neurodegenerative disorders. However, the effect of statins on the major antioxidant pathways in the brain has not yet been investigated. The present study examines the effect of simvastatin on the glutathione antioxidant system in astrocytes. **Methods:** Primary rat astrocyte cultures (15-18 days *in vitro*) were incubated for 3 days with 10µM simvastatin in Dulbecco’s modified Eagle medium containing 10% delipidated foetal calf serum. Cell viability was estimated by Lowry protein assay and/or lactate dehydrogenase (LDH) release. Total antioxidant capacity, total glutathione, and cumene hydroperoxide clearance were measured using colorimetric assays. All experiments used at least two independent cultures (6 wells per condition). **Results:**Incubation with simvastatin for 3d did not cause cell death, nor did it decrease the total antioxidant capacity of the cells. In contrast, total glutathione content was decreased by 25%, compared to controls (37.7±1.6), when cells were incubated with simvastatin (28.6±1.9 nmol/mg protein, $p<0.05$). When simvastatin-treated astrocytes were incubated with cumene hydroperoxide, which can only be detoxified by glutathione, the rate of detoxification was slowed by 22% compared to control astrocytes. However, simvastatin treatment did not increase the toxicity of hydrogen peroxide, even when endogenous catalase activity was inhibited. **Conclusion:** Simvastatin decreases the glutathione content in astrocytes, and slows their rate of peroxide detoxification. Hence statins may not be efficacious in neurodegenerative conditions that involve high levels of oxidative stress.

POS-WED-301

THE HSOD-1 G93A MUTATION INCREASES APOPTOTIC CELL DEATH IN NSC34 CELLS AFTER DEPLETION OF MITOCHONDRIAL GLUTATHIONE

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Purpose: Glutathione, a major endogenous antioxidant, is found in the cytoplasm and in the mitochondria. Depletion of glutathione increases generation of reactive oxygen species and is typically accompanied by oxidative damage and cell death. In the present study, we have tested whether the hSOD-1^{G93A} mutation increases the susceptibility of NSC34 motor neuron-like cells to glutathione depletion and investigated the nature of the cell death that develops. **Methods:** Glutathione was depleted by exposure to ethacrynic acid. Cytoplasm and mitochondria was separated and total (reduced plus oxidized) glutathione was determined spectrophotometrically. Cell viability was assessed using immunohistochemical markers for apoptotic and necrotic cell death. **Results:** There was no significant difference in glutathione content in either pool between the two cell types (n=3). However cells carrying the hSOD-1^{G93A} mutation showed an increased susceptibility to ethacrynic acid (50 µM, 120 min) compared to cells lacking the mutation. Interestingly, this treatment selectively reduced mitochondrial glutathione in both cell types while leaving the cytoplasmic pool essentially intact (n=3). The reduction in mitochondrial glutathione was significantly greater in G93A expressing cells (68.5 vs. 49.8%; n=3). Consistent with these results, cells carrying the hSOD-1^{G93A} mutation showed a 2.8-fold increase in apoptotic cell death in response to ethacrynic acid treatment compared to the normal NSC34 cells (7.0 and 19.8% respectively; n=3). There was no significant difference in necrotic cell death between the two cell types. **Conclusion:** The hSOD-1^{G93A} mutation increases the susceptibility of NSC34 cells to conditions that affects mitochondrial glutathione homeostasis resulting in increased apoptotic cell death.

POS-THU-302

A NOVEL MITOCHONDRIAL DNA (MTDNA) MUTATION ATTENUATES TRANSCRIPTION TERMINATION IN A PATIENT WITH A MITOCHONDRIAL MYOPATHY

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Purpose: To characterise the effect(s) of a novel mutation in the transcription termination region of human mtDNA on transcriptional regulation and respiratory chain activity. **Methods:** Mutation pedigree analysis was done by PCR on DNA extracted from blood and muscle in the proband and blood from his family members. Spectrophotometric analysis of the respiratory chain complexes were performed on lymphoblasts, transmitochondrial cybrid lines and muscle derived from the patient with normal controls (n=6) in each case. Northern blot analysis using probes to the COX1 gene and 18S rRNA was carried out in lymphoblasts, cybrids and controls (n=2 in each case). The full-length mitochondrial transcript, normalised to 18S rRNA, was quantified by real-time PCR on lymphoblasts, cybrids, muscle samples and controls (n=2 in each case). **Results:** The proband had a mutant load of 100% in muscle and 60% in blood. Northern blot and real-time PCR studies showed a significant 2-4 fold increase ($p<0.01$) in the amount of the full-length transcript in the patient's muscle, lymphoblasts and in homoplasmic mutant cybrids. There was a 10-15% increase in Complex I and Complex IV (normalised to citrate synthase) activities in lymphocytes and muscle but not in the cybrids. **Conclusions:** The unique mutation significantly attenuates transcription termination in the human mitochondrial genome. The resulting increase in mRNA appears to elevate respiratory chain activity. This may be a novel molecular mechanism of mitochondrial disease in humans.

POS-WED-303

PHYSICAL EXERCISE IN AGED MICE RESTORES ENDOGENOUS NEURAL STEM CELLS TO YOUTHFUL LEVELS, AUGMENTING THE REGENERATIVE RESPONSE OF THE BRAIN

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While most tissue stem cells undergo age-related alterations, little is known concerning the effects of ageing on neural stem cells (NSCs). To address this shortfall and also provide a benchmark of the effects of normal ageing on endogenous NSCs, we harvested the periventricular region (PVR) from serial vibratome sections through the entire brain(s) of juvenile (6-8 weeks), 6, 12, 18, and 24-month old mice, and cultured equal numbers of cells in the neural colony forming cell assay, which discriminates NSCs from more restricted progenitor cells. While age-related changes in NSC frequency varied along the neuraxis depending upon the rostral-caudal coordinate assayed, as a whole ageing resulted in a series of step-wise declines in NSC frequency starting with a ~40% decline at 6-months, and culminating in a ~90% decline by 24-months. Given that physical activity can alter the age-related decline in spatial memory and hippocampal neurogenesis, we next sought to determine whether a similar regime could slow or reverse the decline in PVR NSCs. Accordingly, 6, 12, 18, and 24-month old mice were given access to a running wheel for 21 days, then sacrificed, and the frequency of resident NSCs determined (as above). NSC frequency significantly increased in runners ≤18-months, but had the opposite effect in 24-month old mice. More importantly, when the regenerative response of 12 and 18-month old mice was assayed by gamma-irradiation induced depletion of the PVR, runners demonstrated an augmented regenerative capacity typical of juvenile mice. Given the lack of an exercise-induced effect on NSCs in growth hormone receptor knockout mice, these studies not only demonstrate for the first time that running stimulates resident NSCs, but also provide the basis for understanding the beneficial effects of running.

POS-THU-304

DIFFUSION WEIGHTED IMAGING AS A MARKER FOR AXONAL PATHOLOGY IN THE INDUCIBLE AUTOIMMUNE MODEL OF EAE:

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Background: Diffusion weighted imaging (DWI) is extensively used to investigate pathology of grey and white matter regions of the central nervous system. Although parallel and perpendicular diffusivities have been shown to alter during diseases such as Multiple Sclerosis, a detailed understanding of the tissue changes underlying these changes has been lacking. **Methods:** We induced MOG 35-55 EAE in C57/BL6 mice, a disease known to affect spinal cord and optic nerves (ON). Mice were monitored for disease progression, and MRI diffusion imaging of the ON, concentrating on the pre-chiasmal optic nerve, was performed on day 16 post EAE induction (n=15). We acquired directional diffusion values parallel and perpendicular to the ON. At this time point, the average EAE severity was (2.4) range (0-3.5). Subsequently, mice were killed with pentobarbitone injection, perfused with glutaraldehyde and optic nerves dissected. After plastic embedding, we cut thin (500 nm) sections and assessed axonal measures including axon number and axoplasmal area using a semi-automated image analysis technique. **Results:** Parallel diffusivity was reduced compared to healthy mice (1.57(±0.14) x10- 3mm^2/s vs1.20 (±0.11) mm^2/s, ($P= < 0.001$). In EAE animals, parallel diffusivity reduction was moderately correlated to axoplasmal area loss (atrophy) within the pre-chiasmal portion of the ON during progressive EAE ($P= 0.1$, $r= 0.2$). Other parameters such as total axon number were poor correlates of diffusivity measures. **Conclusion:** These findings suggest that, using a minimally invasive technique such as MRI, we can assess and monitor the extent of autoimmune axonal injury in the optic nerve during EAE, facilitating the assessment of neuroprotective agents in this animal model.

POS-WED-305

MRI-BASED DIGITAL ATLASES OF THE MOUSE CEREBELLUM AND HIPPOCAMPUS

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Purpose: Construction of atlases of cerebellum and hippocampus of a C57BL/6J mouse. Methods: We used high resolution magnetic resonance microscopy. T2-weighted images, acquired on a 700MHz 16.4T wide bore Bruker microimaging system with a 15mm custom-built birdcage coil. Two 3D gradient echo imaging protocols (Nex=8 and Nex=4) with matrix dimensions = 640 x 394 x 296 were acquired over a 1.95 x 1.2 x 0.9 cm field of view (with 30µm isotropic resolution). The resulting images (from three 8-average scans and seven 4-average scans) were registered, upsampled and averaged together to produce a single high-resolution image. The structures were manually segmented using Display (Montreal Neurological Institute, Montreal, Canada). 36 structures within the cerebellum and 35 structures within the hippocampus were delineated in the three cardinal planes. The mouse cerebellar and hippocampal atlases we have developed provide a detailed segmentation of these structures in a three-dimensional, digital format using magnetic resonance microscopy. Extensions of this approach are likely to be a useful tool in the comparison of diseased models with a control strain. By combining segmentations from a number of animals, the method can also be extended to provide probabilistic information on the size and location of structures within the mouse brain.

POS-THU-306

EDU, A NEW THYMIDINE ANALOGUE FOR LABELLING PROLIFERATING CELLS IN THE NERVOUS SYSTEM

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Labelling and identifying proliferating cells is central to understanding neurogenesis and neural lineages in vivo and in vitro.The standard method of labelling proliferating cells uses the thymidine analogue, bromodeoxyuridine (BrdU), which incorporates into the DNA during S-phase of the cell cycle. A disadvantage of this method is that the immunochemical processing requires pre-treatment of the cells and tissue with heat or acid to reveal the antigen. This pre-treatment reduces reliability of the method and degrades the specimen, reducing the ability for multiple immuno-fluorescence labelling at high resolution. We report here the utility of a novel thymidine analogue, ethynyl deoxyuridine (EdU), detected with a fluorescent azide via the “click” chemistry reaction (the Huisgen 1,3-dipolar cycloaddition reaction of an organic azide to a terminal acetylene). The detection of EdU requires no heat or acid treatment and the incorporated EdU is covalently conjugated to a fluorescent probe, using a copper-catalysed chemical reaction. The reaction is quick and compatible with fluorescence immunochemistry and other fluorescent probes. We show here that EdU efficiently labels proliferating cells of embryos and adult animals. It effectively labels cells during neurogenesis and the progeny may be identified at least 30 days later, thus allowing the tracking and quantification of proliferating cells in multiple neurogenic regions including the olfactory neuroepithelium1. We demonstrate its utility, superseding BrdU as a cell proliferation marker, as it markedly improves the detection of proliferating cells and allows concurrent high resolution fluorescence immunochemistry. 1 Chehrehasa et al 2008, J Neuroscience Methods, In Press.

POS-WED-307

AN EXPERIMENTAL MODEL TO INVESTIGATE CT BRAIN PERFUSION AFTER STROKE

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Purpose: Computed Tomography Perfusion (CTP) imaging of brain post-stroke may allow differentiation of potentially salvageable (“penumbra”) and irreversibly injured (“infarct core”) tissue. It has the potential to significantly improve delivery of acute stroke therapy worldwide. Use of an animal stroke model would enable accurate timing of blood vessel occlusion and reperfusion and correlation of imaging with final histology, and help to validate the method. The aim was to perform pilot studies of CTP imaging rats with experimental stroke, to determine whether images of sufficient resolution could be obtained to correlate with 24 hour histology. **METHODS:** Stroke was induced in male Wistar rats (n=13) by the middle cerebral artery thread-occlusion method and a jugular venous line was inserted. CT scans were obtained on a 64-slice helical CT using a dynamic cerebral perfusion scanning method. Scans were obtained pre- and post-occlusion and hourly for 3 hours. Animals were recovered from anaesthetic and maintained for 24 hours, to allow development of histological changes. Neurological testing was performed for behavioural correlation with the histology and imaging. **RESULTS:** After optimisation of the perfusion and scanning parameters, colour maps were generated for cerebral perfusion (TTP, MTT), flow (CBF) and blood volume (CBV) at multiple coronal planes. The ischaemic region was clearly demarcated on cerebral perfusion maps, and progressive reductions in the CBV within the centre of this region was seen over time. **CONCLUSIONS:** This world-first study has demonstrated the feasibility of performing CTP in the most commonly used animal model of stroke. Definitive studies to determine optimal thresholds and reliability of CTP measures for infarct core and penumbra are in progress.

POS-THU-308

TROPISM AND TRANSDUCTION PROPERTIES OF SEVEN ADENO-ASSOCIATED VIRAL VECTORS IN ADULT RAT RETINA AFTER INTRAVITREAL INJECTION

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Purpose: Recombinant adeno-associated virus (rAAV) vectors are useful vehicles for stable transfer of therapeutic genes to retinal cells. Intravitreal injection is optimal for transduction of retinal ganglion cells (RGCs), although complete selectivity has not yet been achieved. There may also be advantages in using the intravitreal approach for transduction of photoreceptors in the outer retina. Here we compared the tropism and transduction efficiency of seven rAAV serotypes (rAAV2/1, -2/2, -2/3, -2/4, -2/5, -2/6 and -2/8) in adult rat retina after intravitreal injection (n=3-6 animals per vector group). **Methods:** All rAAV serotypes encoded the gene for green fluorescent protein (GFP). For each vector the number, laminar distribution and morphology of transduced GFP^+ cells was determined using confocal and fluorescence microscopy. The phenotype of the transduced cells was assessed by double-immunolabeling with markers for retinal cells. **Results:** rAAV2/2 and rAAV2/6 transduced the greatest number of cells while rAAV2/5 and rAAV2/8 were almost an order of magnitude less efficient. For most vectors, the majority of GFP^+ cells were RGCs, however rAAV2/6 had a more diverse tropism profile. For this vector the proportions of transduced cells were: amacrine and bipolar cells (46%), Müller cells (22%), RGCs (23%). Müller cells were also more frequently transduced by rAAV2/4. The greatest number of GFP^+ photoreceptors was seen after intravitreal rAAV2/3 injection. **Conclusion:** These data will facilitate the design and selection of rAAV vectors to target specific retinal cell types, potentially leading to more effective intravitreal gene therapy for a range of human retinal pathologies.

POS-WED-309

ASSESSMENT OF RESIDUAL FUNCTION AND MORPHOLOGY AFTER SPINAL CORD CONTUSION INJURY IN RATS: BEHAVIOURAL AND HISTOLOGICAL EVIDENCE

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Purpose: Assessment of functional recovery following spinal cord injury (SCI) is difficult because commonly used contusion methods may induce variable incomplete lesions. Because it takes time for recovery from spinal shock and post-operative inflammation and swelling, return of lower limb stepping movements may be misinterpreted as recovery of supraspinal circuitry, particularly when only BBB scores are used. Our study aimed to characterise residual function following recovery from acute SCI compared with the level of apparently intact morphology. **Methods:** Male Sprague Dawley rats (200g) were anaesthetised with Isoflurane, laminectomised and SCI induced at T10 using an impactor. BBB scoring, ledged beam, random rung ladder beam test and digital footprint analysis (Digigait) were tested in rats at 4 and 10 weeks after injury. Following terminal anaesthesia spinal cords were prepared for quantitative morphology. **Results:** In ledged beam and ladder tests there was no significant improvement from 4 to 10 weeks post injury (ledged beam: 9 ± 4 and 7 ± 2 errors; ladder: 15 ± 6 and 12 ± 4 errors, respectively). Footprint analysis parameters were unaltered from 4 to 10 weeks. In contrast, BBB scores indicated recovery of stepping movements between 4 and 10 weeks in SCI rats (7 ± 1 vs 16 ± 2; respectively). None of the morphological measures showed significant changes between 4 and 10 weeks. **Conclusion:** Similarity of 4 and 10-week findings for morphology and most of the behavioural tests suggests there is stabilization of residual function by 4 weeks post-injury. Continued apparent "improvement" in BBB scores alone, should raise concerns about studies in which this is the sole method used for behavioural analysis.

POS-THU-310

MODELLING HYPOTHERMIA AND COMPRESSIVE SPINAL CORD INJURY

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Purpose: Following traumatic spinal cord injury (SCI), spinal cord compression occurs in the majority of patients. Currently, decompressive surgery is performed relatively late because of difficulties organising early surgery and the need to control other life threatening injuries. Persistent compression is not routinely modelled in animal SCI, but can be done and decompression is reported to provide functional recovery (Dimar et al, 1999). **Methods:** We have modified Dimar's method to permit degrees of compression and timed decompression *in-vivo*. After obtaining 2D and 3D Micro-CT images of the rat spine, measurements of the canal cross section between T7-9 were made in three 12 week-old rats. These images were used to create molds for the manufacture of epoxy spacers, designed to compress 35 or 50% of the canal diameter. The epoxy was mixed with red lead for contrast, and monofilament 'tails' were embedded into the distal end, to minimise trauma upon removal. **Results:** Epoxy spacers have successfully created compression in 144 female F344 rats (12-16 weeks) subject to moderate spinal cord contusion (150Kdyne) at T7-9. Mortality is not increased by this procedure, the degree of compression is practical, and the ability to use standard motor function tests (BBB score and the ladder stepping test) are not compromised. We report on the use of this method to assess the effect of hypothermia (33°C) as a tool for reducing the effects of compression, lasting for 0, 2 or 8 hours after SCI. **Conclusion:** Compressive SCI can be successfully mimicked in rats and may be useful for assessment of neuroprotective therapies.

POS-WED-311

DIFFERENTIAL TRANSGENE EXPRESSION FOLLOWING VIRAL TRANSDUCTION IN THE ROSTRAL VENTROLATERAL MEDULLA

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Purpose: Viral gene delivery is a powerful tool for cell-specific transgene expression. The specificity of transgene expression is largely dependent upon viral tropism and the transgene promoter. We used adenoviruses (Ad), lentiviruses (Lv) and adeno-associated viruses (AAV), in combination with cell-specific promoters, to examine transgene expression in the rostral ventrolateral medulla (RVLM). The RVLM contains a heterogeneous population of neurons and astrocytes. **Method:** Four 100 nL microinjections (~$1x10^8$ viral particles/μL) were made into the RVLM of anesthetised male Sprague-Dawley rats (300-500g; n=20). Viruses used in the study were: Ad-cytomegalovirus (CMV)-GFP, Ad-PRSx8-GFP (catecholamine-specific promoter), Ad-Gfa(B)$_3$-lacZ (astrocyte specific promoter), Lv-PRSx8-GFP, Lv-synapsin (Syn)-GFP, AAV-Syn-GFP and AAV-(chicken β-actin (CBA)-tandem dimer Tomato (tdT). One (for Ad) or 4 weeks (for Lv and AAV) post-injection rats were anesthetised, perfused with 4% formaldehyde and brains processed to detect the reporter gene and phenotypic markers. **Results:** Ad-CMV-GFP and Ad-Gfa(B)3-LacZ showed robust transgene expression exclusively in astrocytes. Lv-PRSx8-GFP showed expression in catecholaminergic neurons. Ad-PRSx8-GFP did not show expression in RVLM. Lv-Syn-GFP, AAV-Syn-GFP and AAV-CBA-tdT showed transgene expression in mostly non-catecholaminergic neurons. **Conclusion:** Using different combinations of viral vectors and cell-specific promoters, we have demonstrated selective gene expression in at least three cell subtypes in the RVLM – astrocytes, catecholaminergic neurons and non-catecholaminergic neurons. Interestingly, promoters which are considered ubiquitous (CMV and CBA promoter) or pan-neuronal (Syn) showed cell phenotype-specific gene expression in the RVLM. In conclusion, using a viral vector with a particular promoter will allow us to dissect the contribution of a specific cell population in the RVLM relating to its role in blood pressure regulation.

POS-THU-312

DEVELOPMENT OF A NOVEL IVERMECTIN-GATED CHLORIDE CHANNEL RECEPTOR FOR NEURONAL SILENCING

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Purpose: Reversibly silencing particular neurons should elucidate functions of neurons within a circuit or tune excess neurotransmission in disease. One method is expressing an inhibitory signalling protein and targetting that protein with a selective pharmacological agent. The glycine receptor (GlyR) is a ligand-gated ion channel mediating inhibitory neurotransmission, and the antihelminth ivermectin activates the channel by a novel mechanism. For the purpose of neuronal silencing, we developed a receptor that is insensitive to glycine but sensitive to ivermectin. **Methods:** We used a high through-put fluorescence based assay to screen numerous mutant GlyRs expressed in HEK293 cells, identifying T258S as a mutant that is more sensitive to ivermectin than WT, and we combined this with F207A, which is known to abolish glycine binding. We tested these GlyR mutants with electrophysiology in HEK293 cells and cultured hippocampal neurons. **Results:** In HEK293 cells, F207A was activated by ivermectin (EC_{50}=5.8±0.6 μM; n=4) and not by glycine. T258S showed a 10-fold lower EC_{50} than WT (T258S, 0.08±0.01 μM; WT, 0.7±0.1 μM; n=4). The double mutant F207A/T258S (DM) was activated at lower concentrations than WT, reflected by a lower Hill slope (DM 0.9±0.1; T258S 0.9±0.1, WT 2.2±0.3), although the EC_{50} did not differ from WT (DM 0.8±0.1 μM; n=4). Cultured neurons transfected with the DM showed hyperpolarising currents upon application of 100 nM ivermectin, while WT-transfected neurons showed no currents at such low levels of the drug. **Conclusions:** Our DM receptor is a candidate for neuronal silencing, but we hope to improve its sensitivity to ivermectin by finding more sensitive mutant receptors in our mutant library.

POS-WED-313

AUTOMATED SUBCELLULAR CO-LOCALIZATION IN HCA-VISION

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Of all tools available to study protein-protein interactions, quantitative subcellular colocalisation based on 2D or 3D fluorescence microscopy data arguably provides the most direct information. **Purpose:** In this poster, we illustrate how to perform tailored co-localisation analyses using the subcellular module of HCA-Vision; our High Content Analysis software for studying cell morphology and function. Thus far, co-localisation could only be quantified over the entire cell area, thus reporting on relatively large regions of interest. To address more targeted questions in cell biology, it is necessary to be able to restrict such analyses over well-defined compartments, such as the cell membrane, the nuclei, endosomes, actin bundles, or neurites. **Methods:** The ability to exploit and generate accurate masks using image segmentation is central to the methodology. We have custom developed highly sensitive and readily tunable filters for detecting proteins located in each subcellular compartment. We validate our new approach on several examples, including co-localisation of GLUT4 and VAMPs 2, 3 in adipocyte vesicles. **Results:** The results produced by our software have comparable accuracy with those from semi-automated or manual methods and are obtained at speeds which are orders of magnitude times faster. **Conclusion:** The automated subcellular colocalisation facilitates rapid and accurate evaluation of the spatial localization of two proteins.

POS-THU-314

A PROTOCOL FOR CRYOEMBEDDING THE ADULT GUINEA PIG COCHLEA FOR IMMUNOHISTOLOGY

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Purpose: The structural heterogeneity of tissues in the mammalian cochlea make the collection of well preserved morphological sections challenging. This is particularly true for the adult guinea pig (GP) cochlea, which contains four fluid-filled turns (the most of any mammalian species). Our previous studies identified that transplanted stem cells (expressing green fluorescent protein [GFP]) are not easily distinguished from the high background fluorescence observed after paraffin embedding the GP cochlea. We have therefore developed a protocol for improving the visualisation of GFP-labelled cells within adult GP cochlea, which has the additional advantage of preserving morphology for histological analyses. **Method:** Pre-differentiated GFP positive stem cells were delivered into the adult guinea pig cochlea (n=5) via cochleostomy. Following perfusion, fixation and decalcification the cochleae were cryoembedded so as to preserve both the structure and antigenicity for immuno-analysis and histology. Sections were labelled using anti-GFP and anti-neurofilament antibodies. **Results:** We have developed a protocol that enables the routine collection of 10 µm sections from the adult guinea pig cochlea for both histological and immunohistochemical analyses. Using the described method, we were readily able to identify transplanted stem cells, even after the endogenous fluorescence had faded to background levels. **Conclusion:** Our protocol enables better identification of an authentic GFP signal from innate autofluorescence in the GP cochlea, using either fluorescence-based or chromagen-based immunohistochemistry. It is the first report describing methods to preserve good morphology for immuno-analysis in the adult GP cochlea.

POS-WED-315

POST-MORTEM INTERVAL EFFECTS ON RAT BRAIN USING SELDI-TOF-MS PROTEOMICS

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Purpose: Post-mortem interval (PMI) is one main factor to consider when assessing changes in brain proteins. Applying recently developed new methods of brain tissue preparation for SELDI analysis [direct tissue apposition (TA)[(1)] and paper apposition (PA)[(2)]], we aimed to determine: 1-which PMI condition (time and temperature) resulted in the greatest change to the number of protein peaks, 2-which brain region showed the most changes (was most sensitive to PMI), and 3-the percent homology between the two application methods (TA vs PA). **Methods:** Adult male rats were assigned to one of 8 PMI groups (n=3/group) including body storage at 4°C for 0,6,12,24,48,&72hours, or room temperature (RT; 23-24°C) for 6&12hours. Four brain regions were studied: neocortex, caudate putamen (CP), hippocampus and brainstem medulla. Cryosections from each region were apposed directly (TA) or via the use of Whatman ProteinSaver 903 paper® (PA) onto an NP20 proteinchip, and analyzed by SELDI-TOF-MS. **Results:** Storage at RT resulted in more changes according to PMI than storage at 4°C. At 4°C, PMI>24h resulted in many significant protein changes. Changes were more evident for CP, followed by cortex, medulla and then hippocampus. Compared to baseline, an average of 50% of peaks changed were detected by both application methods, although many more were evident via the TA than PA method. **Conclusion:** Using novel tissue-application SELDI proteomics, we determined that PMI as short as 6h/4°C induced significant changes in a number of protein peaks, and that CP was the region most PMI sensitive, followed closely by the cortex. [(1)]*Bouamrani et al.* (2006) Clin Chem;52:2103-6. [(2)]*Machaalani et al.*(2007) Clin Chem,53:1387-89.

POS-THU-316

IMMUNOPEROXIDASE DETECTION OF NERVES IN FULL-THICKNESS WHOLE MOUNT PREPARATIONS OF HOLLOW ORGANS

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Purpose: Immunofluorescently stained whole mounts have proved useful for defining the innervation of the gut and large blood vessels. Nerves supplying other hollow organs are usually studied in sections, which provide much less information. Aiming to describe the entire sympathetic, parasympathetic and sensory innervation of non-pregnant rat uterus, we developed a method for immunoperoxidase staining of full-thickness whole mounts of uterine horn to visualize all immunoreactive axons. **Method**: Blood was flushed from virgin female rats. Uteri were removed, slit open, stretched, pinned flat and fixed in 4% formaldehyde. Entire uterine horns were treated with methanol/peroxide, washed in Tris-PBS-Triton, blocked in normal serum and incubated in primary and biotinylated secondary antibodies and then avidin-horseradish peroxidase, each for at least 3 days. Metal-intensified peroxidase reactions revealed immunoreactivity. Immunostained whole mounts were dehydrated, infiltrated with resin, mounted on slides under Aclar coverslips and polymerized. Segments of ileum and rectum were treated similarly to determine whether the method was applicable to other hollow organs. **Results**: Non-pregnant uteri contained axons immunoreactive for tyrosine hydroxylase (TH), neuropeptide Y, substance P, CGRP, nitric oxide synthase and the vesicular acetylcholine transporter. These axonal types varied in density and occurred in different layers of the uterine wall and around blood vessels., TH-immunoreactive axons could also be followed through all the layers of whole mounts of ileum and rectum. **Conclusions**: The complete innervation of the uterus and other hollow organs can be revealed by immunoperoxidase staining of full-thickness whole mounts. The resin-embedded tissue does not degrade. The immunostaining is non-fading and permanent. Furthermore, defining the courses of immunoreactive axons that travel between layers does not require time-consuming and expensive confocal analysis.

POS-WED-317

QUANTITATIVE ASSESSMENT OF MISFOLDED HUNTINGTIN CONFORMATIONS IN LIVE CELL MODELS OF DISEASE

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Purpose: In Huntington's disease how the aggregation of mutant huntingtin interferes with cellular functioning and leads to disease is unclear. This problem has been difficult to address because of a lack of methodology for examining protein conformations within living cells. **Methods:** We have been devising new approaches to overcome these limitations with an emphasis on quantitatively detecting the presence and size of the earliest aggregates in simple cell culture models of disease. We have made huntingtin exon 1 reporter proteins that are dual-labeled with two spectrally distinct fluorophores. One fluorophore determines the presence and localization of total protein (cyan fluorescence), while the other determines the presence and localization of a specific conformation (red or green fluorescence). We have also been assessing the oligomeric size of GFP-tagged huntingtin from cell lysates using a fluorescence-adapted analytical ultracentrifuge. **Results:** We have developed two reporter constructs that fluorescently discriminate between distinct conformations of recombinant huntingtin exon 1 *in vitro*. We are currently implementing these constructs into live cells to image the temporal and spatial localization of distinct huntingtin exon 1 conformations. Our analytical ultracentrifuge work suggests that mutant and wild-type huntingtin exon 1 is mostly monomeric in HeLa cells. For the mutant huntingtin-exon 1, inclusions are formed in a highly cooperative nature. **Conclusions:** While this is a work in progress, our strategies offer promising insight into how misfolded huntingtin accumulates and forms inclusions. We anticipate these techniques will enable new approaches to identify how mutant huntingtin affects the cellular machinery in models of disease.

POS-THU-318

DEFINING OLIGODENDROCYTE SURVIVAL FACTORS IN PRIMARY MURINE CELLS

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Purpose: Key survival factors for primary rat oligodendrocytes (OC) were defined more than a decade ago. We have examined survival factors for primary mouse oligodendrocytes using recently described purification methods for these cells (Cahoy et al, J Neurosci 2008). Methods: P7 C57B6 mouse brains were dissected, digested with papain, and immuno-panned through 3 negative selection Griffonia simplicifolia lectin 1Ab coated plates, followed by positive selection using an anti-PDGFRα Ab-coated plate. Yield was 2 x 105 OC progenitors/brain. Cells were expanded in SATO medium with PDGF (5ng/ml), neurotrophin-3 (NT-3, 5ng/ml) and insulin (5µµg/mL). For survival assays, cells were plated (200 cells/well) in differentiation medium and surviving cells counted 68 and 96 hrs, using calceinAM fluorescence, n=20 wells/condition, independent duplicate experiments. Factors were compared to a SATO only condition, including bovine insulin (5µg/mL), Leukemia inhibitory factor (180ng/mL), galanin (1nM), NT3 (5ng/mL), growth-arrest-specific factor 6 (100ng/mL) and combinations of galanin with either NT3 or LIF. Survival in SATO was set at 100%. Results: At 68 hrs, NT3 increased OC survival by 102% (P<0.001), LIF by 67%, P<0.001), Gas6 by 24%, P<0.001), galanin by 41%, P<0.001), combinations of LIF plus galanin and NT3 plus galanin did not increase OC survival above NT3 alone. Morphologically, LIF-treated cells exhibited a more mature phenotype than any of the other conditions. Results at 96 hrs were very similar to 68 hrs. Bovine insulin provided no survival benefit above baseline at either time-point. Conclusion: Many factors known to enhance rat OC survival have similar effects in mouse OC. The effects of LIF were significantly more pronounced in mouse than rat cells. Surprisingly, bovine insulin did not enhance murine OC survival, possibly because it may not engage and signal through murine IGF-1 receptors.

POS-WED-319

MANNOSE RECEPTOR-MEDIATED GENE DELIVERY TO ASTROCYTES

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Purpose: To develop a technique for receptor-mediated gene delivery to astrocytes utilizing the mannose receptor (MR). **Methods:** Immunohistochemical studies were performed in primary cultures of astrocytes prepared from neonatal rat brain and in brain sections from adult Sprague Dawley rats. Confocal microscopy was used to characterise the internalization of a fluorescently labelled MR antibody. In vivo assessment of receptor expression was performed after stereotaxic injections of labelled antibody into the lateral ventricle of anesthetized rats. Finally, the MR antibody was linked to a CMV-driven expression plasmid encoding for green fluorescent protein (GFP), to generate an immunogene used to assess the ability of the MR to facilitate receptor-mediated gene delivery to astrocytes. **Results:** MR expression was detected in > 90% of all cells in primary astrocytic cultures and in the majority of GFAP-positive cells in brain sections (n=4). Intraventricular injection of labelled MR antibody resulted in a selective uptake into periventricular astrocytes (n=3). Internalization of the antibody-receptor complex, as assessed in cell cultures, was rapid and 85.2% of the added antibody was internalized between 2 and 5 min with 43% initially co-localised with clathrin (n=3). Exposure to MR-immunogene (40µg; 3h) resulted in GFP expression in a majority of the cultured astrocytes (80%, n=2) and was competitively inhibited by mannan. No effects on cell viability were observed in the treated cultures. Intraventricular injections of the immunogene (11 µg) resulted in reporter gene expression in astrocytes surrounding the ventricle (n=2). **Conclusion:** This study describes a powerful new approach to selectively manipulate astrocytic function in vivo with the potential to greatly advance the understanding of the contribution of these cells to normal brain function and disease.

POS-THU-320

IMPULSIVE CHOICES IN DOMESTIC CHICKS: EFFECTS OF COMPETITIVE FORAGING AND SSRI (FLUVOXAMINE)

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Purpose: Optimal foragers are predicted to evaluate each food item by its profitability (= amount x proximity). If this is the case, the foragers assume a "small-proximate" food item to be equivalent to a "large-distant" one, when their profitability was identical. In reality, some animals seek for the proximate food item, thus are referred to as impulsive. In order to examine the factors and mechanisms of the impulsiveness, we examined chick behaviors in the inter-temporal choice paradigm. Methods: Chicks were trained to associate colored beads (blue, red and white) with food items (1 or 6 pellets of millet), and were tested in binary choices of beads. The 6- (or 1-) pellets food was delivered after a delay of 0.25~4.51 sec (or 0.1 sec), respectively. Equilibrium point of the delay was determined by behavioral titration everyday for 2 weeks. Results: (1) During the 2 weeks, chicks gradually shifted their choice toward the 6-pellets food. However, from the 5th day onward, the chicks trained competitively with two other individuals had the equilibrium delay that was significantly shorter than those trained in isolation. (2) Systemic injection of SSRI (fluvoxamine, 20 mg/kgBW) significantly shifted chick choices toward the 1-pellet food item. Conclusion: Competitive foraging facillitates impulsiveness through development. Serotonin may be involved in the control of discount rate or time perception.

POS-WED-321

ANALYSIS OF THE NEURAL PROCESSINGS OF THERMO- AND HYGROSENSORY SIGNAL IN THE INSECT BRAIN

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Owing to a large body surface-to-volume ratio, frequent intake of water and locating permissible temperature are particularly important for survival in the insects. Thus, most insects are endowed with the potential to detect minute changes in ambient temperature and humidity. Thermo- and hygroreceptor neurons have been identified on the antennae of a wide variety of insects, including cockroach, *Periplaneta americana*. They are housed in a mushroom-shaped sensillum. Electrophysiological recording from the mushroom-shaped sensillum of a cockroach showed that thermo- and hygroreceptors respond to the cold air and changes of relative humidity, respectively. These receptor cells send their axons to particular sets of glomeruli in the antennal lobe. In our previous study, we identified new classes of projection neurons of which dendrites are confined to these glomeruli and axons project to the particular region of the lateral protocerebrum and the calyces of mushroom body in the protocerebrum. Out-put sites of hygro- and thermosensory projection neurons are obviously distinguished from those of neurons mediating olfactory information. However, how thermo- and hygrosensory signals are processed in the protocerebrum and integrated with other modalities has been totally unknown. Using intracellular recording and staining technique, we successfully identified several types of higher brain neurons, which responded strongly to changes of temperature and/or relative humidity. Some of these neurons possessed dendrites overlapping exactly with output sites of hygro- and thermosensory projection neurons, whereas others had little overlapping with the output sites. These observations indicate that hygro- and thermosensory signals may be processed in two pathways: the specific, unimodal pathway and the multimodal pathway for integration with olfactory signals.

POS-THU-322

HYPOBARIC HYPOXIA INDUCED CA1 DAMAGE AND MEMORY IMPAIRMENT: POSSIBLE THERAPEUTIC STRATEGIES

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Purpose: Calcium plays a crucial role in maintaining cellular homeostasis, however, excess influx of calcium is reported to cause neuronal death via several mechanisms. Exposure to hypobaric hypoxia is known to cause memory impairment however, the mechanisms involved therwith are not yet explored. The purpose of this study was to examine the temporal contribution of L-type calcium channels and N-methyl-D-aspartate receptors (NMDARs) in mediating neuronal death in male Sprague Dawley rats exposed to hypobaric hypoxia simulating an altitude of 25,000 ft for 3, 7 and 14 days. Methods: Behavioral parameters (n=12)-Morris water maze to assess reference memory; Morphology (n=3)- TUNEL, Fluoro Jade-Bstaining, immunofluorescence, immunohistochemistry; Biochemical (n=8)- oxidative stress markers; Protein expression studies (n=3). Results: Blocking of L-type calcium channels with isradipine reduced hypoxia-induced activation of calcium dependent xanthine oxidases, monoamine oxidases, cytosolic phospholipase A2 and cycloxygenases (COX-2) along with concomitant decrease in free radical generation and cytochrome c release. Increased expression of calpain and caspase 3 was also observed following exposure to hypobaric hypoxia along with augmented neurodegeneration and memory impairment which was adequately prevented by Isradipine administration. Conclusion: Administration of Isradipine during hypoxic exposure protected the hippocampal neurons following 3 and 7 days of exposure to hypobaric hypoxia along with improvement in spatial memory.

POS-WED-323

THE DIFFERENT COGNITIVE RESPONSES TO GUQIN MUSIC AND PIANO MUSIC OF YOUNG CHINESE: AN EVENT-RELATED POTENTIAL (ERP) STUDY

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Purpose To further explore whether the psychological effects and neural activation from music of different cultural environments are varied in young Chinese subjects. Guqin and piano music were selected for the experiment. Guqin is the oldest, the most profound art in China and thus the symbol of Chinese civilization. On the other hand, piano is one of the most popular instruments in the West, owning to its immense expressive power. These two instruments are considered to represent two distinctive cultures, Chinese and Western. **Methods** The right-hand young Chinese subjects (mean age is 23 years, SD = 1.34, range 20-29; half males and half females)'s behavioral and event-related potential (ERP) data in the standard two-stimulus auditory (n = 15) and visual (n=13) oddball task were recorded and analyzed respectively. **Result** This study replicated the previous results of culture-familiar music effect on Chinese subjects in auditory task: the greater P300 amplitude in frontal areas in a culture-familiar music environment. At the same time, the difference between guqin music and piano music was observed in N1 and later positive complex (LPC: including P300 and P500): a relatively higher participation of right anterior-temporal areas in Chinese subjects. On the other hand, there is not significant different between Guqin and piano in visual task. **Conclusion** The different effects of Guqin and piano reflected differently in cross-modal (auditory and visual) tasks. And we suggested that the results of the auditory task indicated that the special features of ERP responses to guqin music are the outcome of Chinese tonal language environments given the similarity between Guqin's tones and Mandarin lexical tones.

POS-THU-324

THE EFFECTS OF TOTAL LIGHT DEPRIVATION ON POSTERIOR PARIETAL CORTEX IN RAT NEONATES

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Introduction: Extensive studies, beginning with the pioneering experiments of Hubel and Wiesel have shown that Visual experience during early postnatal life is essential for normal development of visual system. Light Deprivation (LD) during critical periods allows us to investigate effects of LD on Posterior Parietal Cortex (PPC), known as the area for visuomotor control. Materials and Methods: In this study, neonate rats were reared for one month in dark room from 7th postnatal day before beginning of early critical period. A group of rats was taken back into normal condition for 15 days. Animals were transcardially perfused. Coronal sections were prepared from PPC and stained with Nissl, Cytochrome Oxidase and Hoechst to investigate the number of neurons, Volume and Length, Neuronal activity and Hoechst positive cells density with light and florescent microscopy. Results: LD for one month causes loss of neurons, and decreases neuronal activity levels in PPC. Conclusion: It could be concluded that during early postnatal development (Critical Period) of the rat visual system light deprivation causes structural and functional changes in PPC.

POS-WED-325

DISC AUTOTOMY OF THE OPHIUROIDS

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Natalia A. Charlina1, Igor Yu. Dolmatov1, Iain C. Wilkie2 1A.V. Zhirmunsky Institute of Marine Biology FEB RAS, Palchevsky 17, 690041 Vladivostok, Russia, RF; and 2Glasgow Caledonian University, 70 Cowcaddens Road, G4 0BA Glasgow, Scotland, UK Purpose: Echinoderms have an exceptional feature – the presence of mutable collagenous tissue (MCT). This is composed of connective tissue consisting of bundles of cross-banded collagen fibrils accompanied by a loose network of thin fibrils. All MCT structures in ophiuroids share one constant morphological feature, which is the presence of granule-containing cell processes. These processes arise from the cell bodies of juxtaligamental cells, which form a complicated system of ganglion-like clusters on the surface of the ligaments, and also form part of the hyponeural epithelium and basiepithelial nerve plexus of the coelomic epithelium. These cells excrete active substances in response to signals from the nervous system. These substances influence the relationship between extracellular matrix molecules of the connective tissue and change quickly the mechanical properties of the latter. Extreme and irreversible loss of tensile strength in the ligaments and tendons permits autotomy to occur. Results: The ophiuroid is able to detach its central disc from the underlying oral frame in response to external stimuli. Changes can be observed in the ultrastructure of the juxtaligamental components of both the GBL (genital bar ligament) and integument. First the plasmalemma of the juxtaligamental cell processes and the membranes of the intracellular granules in these cells break down. The cell processes become depleted of granules and their contents appear to be dispersed in the extracellular compartment. At the same time the whole integument is disrupted. In addition, the GBL and integumental connective tissue becomes disorganised with the collagen fibrils separating from each other as if there had been a loss of interfibrillar cohesion. This must result in the catastrophic loss of tensile strength of both the GBL and integument. The GBL tears apart approximately half way between the genital bar and lateral arm plate.

POS-THU-326

RESPONSE OF TERMITE ANTENNAE TO ENTOMOPATHOGENIC FUNGAL ODOUR

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Purpose Recently biological control with entomopathogenic fungi to termite has been studied as a possible alternative to chemical control. Since termites live in high density population and a high humidity habitat which has suitable properties for fungal infection, fungal diseases can be quite lethal. However, it is difficult to find fungal epizootics in termite populations. Several researches try to give the explanation, but there is not enough information about the nature of the relationship. Therefore, our purpose of this study is to illustrate clearer the interaction between pathogenic fungi and termite. **Methods** It is well known that termites, *Coptotermes formosanus* Shiraki, protect themselves from entomopathogenic fungi by mutual grooming behaviour. To know the fungi-termite interaction, we examined the role of antennae in grooming behaviour using electrophysiological methods since antennal contact is important as introduction of other social behaviours. **Results** Here, we report the behavioral change of termites against fungal odour, electroantennogram (EAG) and single sensillum responses to fungal odour and the number and distribution of identified sensillum, which responds to the odour. **Conclusion** Our results suggest that termite can detect the entomopathogenic fungi using olfactory receptors on antennae. However, it seems that odour is not the only cue to detect fungi.

POS-WED-327

A NOVEL P 75NTR MEDIATED MECHANISM PERTAINING TO GLUTAMATE INDUCED NEURODEGENERATION IN HYPOBARIC HYPOXIA

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Purpose: Hypobaric hypoxia has been reported to mediate oxidative stress, neuronal degeneration and dendritic atrophy in the hippocampus along with impairment in memory. Glutamate in particular, has been reported to play a key role in mediating excitotoxicity in hypoxic conditions primarily through mitochondria mediated apoptotic cascades and free radical generation. The present study therefore aimed at exploring the molecular events pertaining to glutamatergic transmission following exposure to hypobaric hypoxia simulating an altitude of 25,000ft for 3 days, 7 days and 14 days. Methods: Behavioral studies (n = 15 per group) were conducted in the Morris Water Maze following which the animals were sacrificed. Histological (n = 6 per group), biochemical (n = 6 per group) and molecular parameters (n = 3 per group) were conducted to study neurodegeneration, oxidative stress and protein expression. Results: Our investigations revealed elevation in levels of synaptic glutamate along with increased intracellular calcium following exposure to hypobaric hypoxia. This along with upregulation of NMDA receptor expression and downregulation of GluR2 subunit of AMPA receptors resulted in excitotoxic cell death following hypobaric exposure. In addition to the classical mitochondria mediated apoptotic cascades triggered by release of cytochrome c due to calcium overload, a novel p75 NTR mediated apoptotic cascade was also discovered. Increased p75NTR expression through a NMDAR mediated mechanism was observed. Silencing of p75NTR on the other hand decreased neuronal apoptosis following hypoxic exposure. Conclusion: Our study therefore reveals operation of a novel p75NTR mediated apoptotic cascade in excitotoxic conditions in addition to the mitochondria mediated apoptotic cascade. These findings have a therapeutic implication for neurodegenerative diseases associated with hypoxia.

POS-THU-328

THE CENTERING RESPONSE IN FLYING BUDGERIGARS *(MELOPSITTACUS UNDULATUS)*

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We investigated whether and how birds fly through narrow gaps by filming the flights of budgerigars using high speed cameras, as they flew through an indoor tunnel. The walls of the tunnel were either blank, or decorated with black-and-white stripes. When the walls were blank, the birds tended to fly through the middle of the tunnel. The same was true when both walls carried vertical stripes. However, when one wall carried vertical stripes and the other wall was blank, the birds flew at a large distance from the striped wall and tended to crash into the blank wall. When one wall carried vertical stripes and the other horizontal stripes, the birds flew close to the wall with the horizontal stripes. These findings suggest that, flying budgerigars negotiate narrow spaces by positioning themselves so as to balance the speeds of image motion that are experienced by the two eyes. This strategy enables them to fly safely through narrow gaps in a collision-free manner, provided the walls on the two sides offer image motion cues (optic flow). Budgerigars have laterally positioned eyes with a small region of binocular overlap. However, binocular stereopsis does not seem to play a role in this centering behavior. Our findings reveal interesting parallels with visually guided behaviour in flying insects such as honey bees, which also display a centering response that is mediated by optic flow cues. In most insects, the small interocular separation does not permit stereo-based ranging of distant objects or surfaces.

POS-WED-329

MOVING IN THE DARK: VISION AND NAVIGATION IN THE BULL DOG ANT *MYRMECIA PYRIFORMIS*

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Purpose: We report on the navigational strategies and visual systems of the crepuscular bull dog ant *Myrmecia pyriformis*, a solitary foraging species that heads to nearby eucalyptus trees to forage. **Results:** We find that *M. pyriformis* begins activity during the dusk twilight, with foragers leaving the nest in one burst shortly after sunset. Being active at night places pressures on their visual systems and so requires physiological and behavioural adaptations to allow them to exploit their low light environment. We find that their compound eyes are well adapted to low light levels, with large facets and large rhabdoms increasing light sensitivity. Also, behavioural evidence indicates that they may be employing temporal summation, a process that increases visual reliability by integrating signals over longer periods of time. Ants are known to use a number of strategies to navigate, among them the use of polarised skylight and the visual panorama. We placed a sheet of polariser in the paths of foraging ants and asked whether they responded differently to polariser orientations 45° either side of the dominant direction of skylight polarisation at dusk. We find that the ants do indeed notice the change in the direction of skylight polarisation. In addition, we find that the ants are disoriented when we block their view of the landmark panorama with a large screen. Bulldog ants thus use multiple navigational cues during foraging.

POS-THU-330

THE EFFECT OF SOCIAL STRESS ON TYROSINE HYDROXYLASE PHOSPHORYLATION

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Purpose: Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, is regulated acutely by protein phosphorylation (secs-mins) and chronically by protein synthesis (days). Previous findings suggest the involvement of TH in response to different types of stressors. In these studies we aimed to investigate for the first time the phosphorylation of TH occurring within the first 24 h in response to the social conflict stress. **Methods:** TH phosphorylation was studied using the rodent social conflict model. Animals (intruders) were subjected to the social conflict stress by placing them into the cage with a resident. Intruders were then sacrificed 10 min or 24 h after the social conflict. Adrenals and brains were removed and TH phosphorylation and TH protein were analysed by western blotting. **Results:** We found that in adrenals pSer40 in TH in response to the social conflict was not changed after 10 min, but was significantly decreased after 24 h (1.75 fold, $p<0.05$, $n=6$), while pSer19 and pSer31 were not changed at any time. In substantia nigra no changes in TH phosphorylation were observed after 10 min or 24 h. In ventral tegmental area pSer31 was increased after 10 min (1.5 fold, $p<0.05$, $n=6$) but not after 24 h. In locus coeruleus pSer40 was significantly increased after 10 min (1.35 fold, $p<0.05$, $n=6$), but not after 24 h. **Conclusions:** We provide evidence for the first time that the social conflict stress differentially affects TH phosphorylation in adrenals and different brain regions after 10 min and 24 h which may have a significant role in stress response.

POS-WED-331

COMPETITIVE INHIBITION OF GABAA RECEPTOR GAMMA2 IL2 BINDING REDUCES SINGLE CHANNEL CONDUCTANCE

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Recombinant GABAA receptors (GABARs) have single channel conductances between ~11-35 pS, but higher conductances, >40 pS, were reported for recombinant GABARs co-expressed with a GABAA receptor associated protein (GABARAP)[1]. Neuronal GABAA channels activated by GABA and potentiated by drugs such as the benzodiazepine subunit-containing GABARs γ2 diazepam, also show channels with conductances >40 pS (HC)[2]. A 23-amino acid motif in the MA region (γ2 MA) of the intracellular loop (IL2) of the γ2L subunit has been shown to interact with the intact γ2L subunit[3]. **PURPOSE**: We hypothesised that self-interaction between two γ2 subunits involving the γ2 MA motif is a possible means of achieving co-ordinated responses between GABARs that result in apparent HC channels. **METHODS:** Using inside/out patches, the γ2 MA peptide (rat γ381-403) was applied to the cytoplasmic surface of cultured hippocampal neurons and recombinant α1β1γ2 GABARs. **RESULTS**: Following exposure of neuronal GABARs to the γ2 MA peptide (1-25 μM), the weighted-mean conductance of HC GABA-activated chloride channels was significantly reduced from 48±2.5 pS to 35±1.5 pS ($n=15$; $p<.01$); there was little change following exposure to a randomised γ2 MA peptide (47±0.94 pS, 44±1.1 pS, respectively, $n=15$). A similar decrease in single channel conductance was observed in neuronal HC channels following potentiation by diazepam ($n=16$) and likewise of recombinant α1β1γ2 channels that had been co-expressed with GABARAP ($n=4$). **CONCLUSION**: GABAR HC channels could arise through inter-receptor interactions via the γ2 subunit IL2 loop. 1. Everitt et al., J Biol Chem 279, 2004. 2. Eghbali et al., Nature 388, 1997. 3. Nymann-Andersen et al., J Neurochem, 80, 2002.

POS-THU-332

THE NEUROTROPHIN BDNF DIFFERENTIALLY AFFECTS SURVIVAL AND OUTGROWTH OF MOUSE TYPE I VS TYPE II SPIRAL GANGLION NEURONS

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In the cochlea, the majority of the spiral ganglion neurons (type I) innervate inner hair cells, while a small subpopulation (type II) innervate the outer hair cells in an *en passant* fashion. This innervation pattern is established in the first post-natal week in rodents. **Purpose:** To test for possible differential actions of neurotrophins on these two populations of spiral ganglion neurons during the developmental window when the afferent reorganization is occurring. **Methods:** Spiral ganglion explants were prepared from neonatal mouse cochleae at postnatal day 1 and day 7. Type I and type II spiral ganglion neurons were distinguished by comparing immunolabelling with ß-tubulin, which labeled all neurons, with peripherin immunofluroescence, which only labeled type II neurons. **Results:** Analysis showed that after two days in culture there was significantly greater loss of type I vs type II neurons in control conditions lacking neurotrophin support. When brain-derived neurotrophic factor (100ng/ml BDNF) was added to the media, Type I neuron survival was enhanced. However, BDNF was effective at promoting more neurite outgrowth in both type I and type II neuron populations.,**Conclusion:** Type I and type II spiral ganglion neurons are differentially affected by neurotrophic support during the period when their hair cell targets are being selected.

POS-WED-333

INCREASED GLUA1 AND GLUN1 LEVELS IN THE DENTATE GYRUS MIDDLE MOLECULAR LAYER 48 HOURS AFTER LONG-TERM POTENTIATION AT PERFORANT PATH SYNAPSES IN VIVO

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Long-term potentiation (LTP) of synaptic transmission can persist for days or longer in the rodent dentate gyrus in vivo and is widely accepted as a cellular mechanism of information storage. While the short-term mechanisms of LTP have been well described, biochemical correlates of long-lasting LTP have not been established. Previously, we have shown late-LTP associated increases in both α-amino-3-hydroxy-5-methyl-4-isoxazole receptor (AMPAR) and N-methyl-D-aspartate receptor (NMDAR) expression. However, synapse-localised changes were not observed for AMPAR-subtypes, possibly being occluded by nonpotentiated synapses, or homeostatic mechanisms. Here, laser microdissection has been used to isolate stimulated and non-stimulated zones of the dentate gyrus molecular layer 48 h following induction of perforant path LTP in awake animals. High-frequency stimulation to the medial perforant path induced LTP at middle molecular layer (MML) synapses (48 h extracellular field potential: 20 ± 3% of baseline, n=8), and heterosynaptic LTD at outer molecular layer (OML) synapses (-24 ± 4%, n=8). Expression of NMDAR subunit GluN1 was elevated in the MML 48 post-LTP (12 ± 5%, n=8, $p<0.05$, Student's t-test), but not the OML, or inner molecular layer. Relative distribution of AMPAR subunit, GluA1, was increased in the MML at 48 h (LTP: middle layer 153 ± 15 % of inner layer; control: middle layer 112 ± 11 of inner layer, n=7, $p<0.01$, two-way ANOVA). GluA2 expression was unchanged. Together these data suggest that a molecular signature of late-LTP may be enlargement of postsynaptic specialisations, with a larger GluA1-containing AMPAR population.

POS-WED-335

O6-METHYLGUANINE-DNA METHYLTRANSFERASE(MGMT) IN HUMAN GLIOMAS

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The deoxyribonucleic acid(DNA) repair gene, O6-methylguanine-DNA methyltransferase(MGMT), is a well-known methyltransferase to induce resistance to alkylateing agents such as Temozolomide(TMZ). We analysed promoter methylation of MGMT in 9 human glioma patients and tested the relationship with cyclin-dependent kinase inhibitor 2A/B(CDKN 2A/B) methylation and phosphatase and tensin homolog(PTEN) and the tumor suppressor gene(TP53) mutation. In this study, we demonstrate the association with the promoter region methylation or gene mutation and the outcome of patients in human glioma.

POS-THU-336

NPAS4 HAS A ROLE IN $GABA_{ERGIC}$ NEUROGENESIS

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Purpose: NPAS4 is a basic-Helix-Loop-Helix / Per-Arnt-Sim transcription factor expressed in the neurogenic regions of the brain. In the adult brain NPAS4 is known to be induced by seizure, cerebral ischemia or cortical depolarisation. Here we investigate the developmental role of NPAS4 in the brain. **Methods:** Knock-down of NPAS4 expression was achieved during embryogenesis in *Danio rerio* by injection of antisense translation-blocking morpholino oligonucleotides. Injections were performed at the 1-2 cell developmental stage. Typically 50-100 embryos were injected with control or specific morpholinos with each experiment. **Results:** NPAS4 expression was detected in the embryonic brain of the zebrafish in discrete regions of the prosencephalon and diencephalon. Knock-down of NPAS4 expression during embryogenesis produced a specific morphant phenotype typified by a reduced brain size and abnormalities forebrain development. Patterning of the diencephalon was perturbed most likely as a result of reduced sonic hedgehog expression in the zona limitans intrathalamica. Reduced expression of NPAS4 also led to anomalies in GABAergic neuron production in the forebrain as revealed by reduced dlx1 expression. **Conclusions:** NPAS4 has an important transcriptional role in forebrain development and GABAergic neurogenesis. NPAS4, like other members of the bHLH/PAS family, has a due role; in development of the forebrain and neurogenesis, as well as, responding to pathophysiological stresses by the mature brain.

POS-THU-334

RECEPTOR-ASSOCIATED PROTEIN (RAP) BINDS TO β-AMYLOID (Aβ) AND MODULATES ITS AGGREGATION, CELL BINDING, AND NEUROTOXICITY

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Alzheimer's disease (AD) is characterised by an accumulation of amyloidogenic β-amyloid protein (Aβ) in the brain. As binding of Aβ to the plasma membrane may be a critical event in AD, we investigated the binding of fluorescein-labeled $Aβ_{1-42}$ ($FluoAβ_{1-42}$) to SH-SY5Y neuroblastoma cells. The cells bound and internalised $FluoAβ_{1-42}$ over several hours. Since Aβ is reported to bind the low-density lipoprotein receptor-related protein (LRP1), we examined the effect of an endogenous LRP1 ligand, receptor-associated protein (RAP), on the Aβ-cell interaction. We found that over a 4-6 h time course, the cell binding of $FluoAβ_{1-42}$ was enhanced by RAP co-incubation. $FluoAβ_{1-42}$ and RAP co-localised when bound to the cell surface. However, the binding of $FluoAβ_{1-42}$ to cells, in the presence and absence of RAP, was independent of LRP1. We found that RAP bound directly to Aβ, and formed an SDS-stable complex. The RAP-Aβ interaction inhibited $Aβ_{1-40}$ oligomerisation and $Aβ_{1-42}$ fibril formation. Next, the functional consequences of the RAP-Aβ interaction were examined. An $Aβ_{1-42}$-induced increase in intracellular Ca^{2+} was inhibited by co-treatment with RAP. In addition, we found that RAP co-injection blocked an Aβ-induced inhibition of memory consolidation in day-old chicks. Together, these results demonstrate that the interaction of RAP and Aβ alters Aβ aggregation and cell binding. This RAP-Aβ interaction described here may underlie RAP's inhibition of Aβ-induced effects *in vitro* and *in vivo*. Our findings implicate the RAP-Aβ interaction as a potential modulator of the AD pathological cascade.

POS-WED-337

THE EFFECT ON ABDOMINAL MUSCLE TRAINING ON COUGH AFTER SPINAL CORD INJURY

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Purpose: Respiratory complications are the major cause of death of people with high-level spinal cord injury (SCI, impairment level ≥T5) because they have a reduced ability to cough due to abdominal muscle paralysis. We investigated the effect of cough training on coughing ability using functional electrical stimulation of the abdominal muscles using a novel positioning of stimulating electrodes that enhances cough (1). **Methods**: 15 subjects with SCI (C4-T5) were trained for 6 weeks, 5 days/week (5 sets of 10 coughs/day) using a cross-over design. Subjects coughed voluntarily at the same time that a train of electrical stimulation was delivered (50 Hz, 1s). Measurements were made of expiratory abdominal and thoracic pressures during a cough with a gastro-oesophageal catheter and expiratory flow and volume. **Results**: After training, no differences were found in 1) the evoked maximal abdominal muscle twitch pressure, 2) the stimulus intensity of the train of stimuli required to produce an abdominal pressure of $40cmH_2O$ at rest, or 3) the peak expiratory flow, expired volume and expiratory pressures produced in stimulated coughs. However, during unstimulated voluntary coughs, abdominal and thoracic pressures increased after training but this did not translate into an increase in the peak expiratory flow or expired volume. **Conclusion**: Cough training may be beneficial to SCI subjects to increase the expiratory pressures that can be produced during voluntary coughs.
1. Lim et al. (2007) *J Appl. Physiol.* 102,1612-1617.

POS-THU-338

SHAPE DISCRIMINATION IN REEF FISH

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Purpose: Coral reef fish live in a complex world of colour and patterns. If they are to survive they need to be able to tailor their behaviour to suit the things they see. For example, they need to be able to discriminate between things that are good and bad to eat, and predators from potential prey. Carrying out this task is far from trivial and will draw on a range of cues spanning many sensory modalities. Here we focus on the visual abilities of a reef damselfish, *Pomacentrus amboinensis*. **Methods:** In five experiments, two groups of fish (n=10, n=8) were trained on either a 3-dimensional or 2-dimensional circle, and tested against various distracter stimuli. Initially (exp1-4), the reward was directly associated with the target so that contact with the target led to the immediate release of food from the attached feeding apparatus. In experiment 5, the reward was separated from the target in both space and time to exclude any olfactory cues. **Results:** Group 1 learned to associate their rewarded stimulus (circle) with a food reward within 12 sessions (6 days) of capture and were able to discriminate the circle from a rectangular target that was volume (exp1), width (exp2) or width and height (exp3&4) matched within 10 testing sessions each. Group 2 (exp5) was not only able to discriminate more complex stimuli (windmill, circle) within the same time frame but also showed anticipatory behaviour. **Conclusion:** Freshly caught reef fish are not only able to quickly learn and discriminate novel stimuli in a number of different conditions but are also able to interpret stimuli as predictor for the availability of food at a different time and place.

POS-WED-339

EFFECT OF ANTIOXIDANTS IN PACEMAKING OF MICE LOCUS COERULEUS NEURONS

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The locus coeruleus (LC), a nucleus in the brain stem pons, contains tightly packed noradrenergic neurons. This group of neurons correspond to the biggest noradrenergic source in the brain, extending projections to most brain areas. We have been studying the rhythmicity of these neurons and aspects such as the role of mitochondria and free radicals in this process. Purpose: The study reported here is an investigation into the impact of different antioxidants in the signalling produced by free radicals on the interspike-interval pacemaker currents in mouse LC neurons. Methods: Experiments were conducted using in vitro brain-stem slice preparations at 37oC and whole-cell patch-clamp recordings. Mice were euthanased by decapitation, a method approved by the Animal Care and Ethics Committee at the University of Newcastle. Two different antioxidants were used (vitamin E analogue Trolox and selective –SH group antioxidant DTT) along with drugs to disrupt mitochondrial activity (CCCP). Results: Trolox by itself did not have any measurable actions (n=4), but DTT had a small effect on pacemaking (n=8). Trolox combined with CCCP had two effects: First it increased the outward current induced by CCCP in the interspike interval range, and second it reduced CCCP-induced current at membrane potentials more positive than -40mV (n=12). Both DTT (n=9) and Trolox (n=12) reduced the inhibition of action potential firing induced by CCCP. Conclusion: These results indicate that mitochondria release free radicals that in turn have a role in the pacemaking process. The roles of specific K channels in these actions are under investigation.

POS-THU-340

GANGLION CELL TOPOGRAPHY AND RETINAL RESOLUTION OF THE SLEEPY LIZARD (SCINCIDAE: TILIQUA RUGOSA)

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The Australian sleepy lizard (Scincidae: *Tiliqua rugosa*) occupies stable overlapping home ranges and maintains long-term monogamous relationships. Such associations require accurate inter-individual recognition and, amongst other cues, sleepy lizards use visual information to accomplish this task. In this study we evaluated the total number, size, topographic distribution, and density of ganglion cells in the sleepy lizard retina. Identified by their size and shape, prominent nuclei and the accumulation of Nissl-positive substances in their cytoplasm, ganglion cells were easily discernable from amacrine and glial cells. The topographic distribution of ganglion cells revealed a horizontal band of high cell density in the central retina. This area, located approximately one mm dorsal of the optic disc, may correspond to the visual streaks widely observed in mammals. Within the streak, ganglion cells peaked at a mean density of 15,500 cells/mm^2. With a posterior nodal distance of 6.30 mm, the retinal resolution was estimated to be 6.9 cycles/degree. This is the first anatomical estimation of visual acuity in a lizard species.

POS-WED-341

CHROMATIC AND LUMINANCE CONTRAST IN VISUALLY GUIDED REACHING

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Purpose: The dominance of fast monochromatic visual pathways in conveying motion information along the dorsal stream in primates is well established. But primates have evolved slower pathways for additional colour processing. The respective roles of chromatic and luminance information in updating changes in locations is unclear. Brenner and Smeets (2003) reported that corrections to reaches towards chromatic targets are almost as fast as corrections to luminance defined targets. However, it is likely that their equiluminant targets also stimulated the fast pathway, reducing the apparent difference between the two target types. We compared delays in corrections to reaches to chromatic and the monochromatic targets that suddenly moved to determine the varying contributions of the two visual pathways in updating reaching movements. **Methods:** Equiluminance and salience were determined subjectively for each participant. Then, participants focused on a fixation cross and initiated each trial with a key press. In 3 out of 4 trials, the cross turned into a dot that participants had to reach too. In 1 in 4 trials the dot appeared 6 degrees above the cross at a random time during the reach. The dot was either luminance-defined or chromatically-defined at random. The correction in the higher dot's direction and the time of exposure to the dot were recorded. **Results:** Time of exposure to the higher target's location was correlated with the percentage of correction in 2 observers. For both luminance-defined and chromatic stimuli, observers displayed a greater correction when they had more time to compensate. For this range of exposure times, there was generally no significant difference in correction for chromatic or luminance defined stimuli. **Conclusion:** The delay in correcting online reaches to chromatic and luminance defined targets is similar.

POS-THU-342

MULTIMODAL SIGNALING IN FOWL, GALLUS GALLUS

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Purpose: Food calling is widespread in social birds and primates. In galliforms, these vocalizations are typically accompanied by a distinctive visual display, creating a multimodal signal known as tidbitting. This system is ideal for experimental analysis of the way in which display components interact to determine signal efficacy. **Methods:** We used high-definition video playback to explore perception of male tidbitting by female fowl, *Gallus gallus*. In this experiment, females (n=24) experienced four treatments consisting of multimodal tidbitting, visual tidbitting without sound, audible tidbitting without a male present, and a silent empty cage control. **Results:** Hens took longer to begin food search when the display was silent, but the overall rate of this response did not differ among the multimodal, visual only or audio only playback treatments. These results suggest that the tidbitting is a redundant signal, but they also highlight the importance of a temporal dimension for any categorization scheme. Visual displays also evoked inspection behavior, characterized by close binocular fixation on the head of the playback male. This is known to facilitate individual recognition. It may also allow hens to assess male quality. **Conclusion:** Such social responses reveal that tidbitting likely has multiple functions and provide a new insight into the selective factors responsible for the evolution of this complex multimodal signal.

POS-WED-343

NEONATAL LIPOPOLYSACCHARIDE EXPOSURE PRODUCES LONG-TERM ALTERATIONS TO NEUROIMMUNE AND NEUROENDOCRINE FUNCTIONING IN THE RODENT

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Purpose: Neonatal stress exposure coupled with adult stress has been demonstrated to produce perturbations in HPA axis functioning. This 'two-hit' model suggests that perinatal insults can produce a physiological vulnerability in later life, however, the central mechanisms governing these changes remain unclear. We examined the impact of neonatal immunological stress coupled with adult psychological stress on the neuroendocrine-immune response to stress. Methods: Wistar rats were administered lipopolysaccharide (LPS; salmonella enteritidis, 0.05mg/kg, n = 100) or saline (equivolume, n = 70) intraperitoneally on postnatal days 3 and 5. Trunk blood was collected from a subset of animals (n = 20) to assess neonatal corticosterone responses to LPS administration. In adulthood, animals were allocated into either a "stress" (30 minutes restraint) or "no stress" condition. Blood was collected at baseline, 30 and 60 minutes to assess serum corticosterone concentrations. Animals were euthanised 2 hours post-baseline and brains were collected to assess 1) hippocampal concentrations of interleukin-1β (IL-1β); or 2) c-Fos staining of limbic structures. Animals were perfused with saline, and hippocampal tissue was rapidly dissected and snap frozen for IL-1β analysis using ELISA. c-Fos was assessed in brains extracted from animals perfused with 2% sodium nitrite and 4% formaldehyde following either adult treatment condition. Results: Neonatal LPS exposure significantly increased corticosterone levels on day 5 following injection compared to controls ($p < .05$). Neonatal LPS coupled with adult stress produced significantly higher serum corticosterone and hippocampal IL-1β concentrations compared to all other groups ($p < .05$). Cell counts of c-Fos staining revealed significantly greater activation in the cornu ammonis and dentate gyrus of the hippocampus, and in the paraventricular nucleus for neonatally LPS-treated animals compared to their control counterparts. Conclusion: The data indicate that early life infection can permanently alter the neuroendocrine response to stress and that central IL-1β signalling pathways may mediate this effect. Furthermore, cFos staining revealed that neonatal LPS exposure produces a long-term neural propensity for maladaptive stress responsivity.

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HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP-1) IS A NOVEL REGULATOR OF EXOCYTOSIS

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Purpose: Huntington's disease (HD) is a fatal neurodegenerative disorder the caused by a mutation in the Huntingtin (Htt) gene. Htt binds to and localises intracellularly with Huntingtin-Associated Protein 1 (HAP-1) and HAP-1 has a greater binding affinity for mutant Htt. HAP-1 is expressed particularly in areas affected in HD and may therefore be important in HD pathogenesis. As HAP-1 and Htt are thought to play a role in microtubule transport our aim was to investigate whether HAP-1 regulates an end-stage of transport, vesicle exocytosis. Methods: Adrenal chromaffin cells used for our exocytosis assays were cultured from day old HAP-$1^{-/-}$ (KO), HAP-$1^{+/-}$ (Het) and HAP-$1^{+/+}$ (WT) mice. Amperometry was used to measure exocytosis in single cells and real-time RT-PCR used to measure mRNA expression. Results: WT (102.9 ± 13.4 exocytotic events, n= 25) and Het (91.3 ± 10.9, n=21) cells display similar exocytosis levels whereas exocytosis in KO cells is significantly reduced (60.1 ± 6.9, n=36) compared to WT ($p<0.01$) or Het ($p<0.05$). Analysis of individual amperometric spike shapes illustrates differences in the pre-spike "foot signal", an indicator of fusion pore stability and formation. Foot duration is prolonged in KO (2.09 ± 0.22 ms) compared to WT (1.55 ± 0.12 ms, $p<0.05$) and Het (1.45 ± 0.08 ms, $p<0.05$) cells. The size of the readily releasable pool is also reduced in KO cells as is the expression of several exocytosis genes. Conclusion: Our findings illustrate a previously unknown role of HAP-1 in regulating exocytosis at multiple stages including trafficking, fusion and transcription. If HAP-1 similarly affects neurotransmission then the potential exists for an involvement of HAP-1 in HD pathogenesis.

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A COMPARISON BETWEEN THE SOFTWARE-BASED AND MANUAL RECORDINGS OF SOCIAL ENCOUNTERS IN RODENTS

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Purpose: Schizophrenia is a psychiatric condition which is characterized by positive and negative symptoms. Positive symptoms include delusions, hallucinations and cognitive dysfunction. Negative symptoms include alogia, avolition and social withdrawal. In preclinical studies, social withdrawal is assessed in rodents using the social interaction test. The test involves recording the number of social encounters between unfamiliar animals. This recording can be performed either automatically using computer software such as EthoVision® and SMART® or manually by an unbiased observer. To date, no comparisons have been made to determine which method produces results that show the highest level of resolution between the effects of drugs that induce social withdrawal, such as dizocilpine (MK-801), and vehicle in rodents. Methods: Male Sprague-Dawley rats (150-250 g) were housed in groups of 6 for 5 days prior to social interaction testing in the open field. On day 6, the rats were injected with either vehicle or 0.6 mg/kg MK-801. They were then tested in the open-field for 10 minutes with unfamiliar conspecifics. Each test session was videoed and later analysed by SMART® or by an unbiased observer. Results: The software was unable to distinguish between the effects of MK-801 and vehicle on social interaction. However, the observed-based recording showed a clear effect of MK-801 in inducing social withdrawal in the rats. Conclusion: These findings suggest that, unlike the manual approach, the software-based method of recording social encounters is unable to differentiate between passive and active interactions.