POSTERS

POS-TUE-001

THE INITIAL AXON OUTGROWTH FROM THE OLFACTORY EPITHELIUM

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The olfactory system provides an outstanding model that allows for the understanding of the mechanisms that drive neurodevelopment and axon-glia interactions. This system is unique because new neurons are constantly generated from stem cells that line the basal layer of the olfactory epithelium. New axons then extend from the epithelium into central nervous system where they terminate. The glia of the olfactory system, the olfactory ensheathing cells (OECs), are thought to be essential for the regenerative capacity of the olfactory system. However the initial outgrowth of axons and the interactions with OECs during early development are poorly understood. To visualise olfactory axons in early development we used OMP-ZsGreen transgenic mice at the ages E10.25 to E13 (n=3 at each age). The bright fluorescence of the ZsGreen enabled us to view growth cone morphology and track the trajectory of the axons as they exited the basal layer of the olfactory epithelium and projected into the central nervous system. Using the ZsGreen axons, combined with a more sensitive immunohistochemistry protocol, we have identified that olfactory sensory neurons first arise at E10.25 and their axons penetrate the telencephalon at E11.0. At E10.75 we have also identified the presence of dendrites projecting from the olfactory neurons. OECs migrate ahead of the axons and establish the pathway through which the axons extend which can be seen from as early as E11. These results demonstrate that the establishment of the olfactory nerve pathway is dependent on the migration of OECs and that olfactory axons penetrate the olfactory bulb earlier than previously thought.

POS-TUE-002

ALTERING DOPAMINE ONTOGENY IN DROSOPHILA MELANOGASTER INCREASES VISUAL RESPONSIVENESS IN ADULT MALES

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Purpose: Epidemiological evidence indicates that schizophrenia is a neurodevelopmental disorder. At a neurochemical level, it would appear that there are also underlying abnormalities in dopamine (DA) signaling in the brain as a result. Therefore the aim of this research, was to use the invertebrate model *Drosophila melanogaster*, which provides genetic tools to exquisitely control spatial and temporal expression of genes, to investigate the effects of transiently altering DA activity during early fly development and measure the effects of such alterations on visual behaviour of adult flies. **Methods:** DA signaling was manipulated to either attenuate or potentiate DA release during four critical developmental epochs in fly brain development. DA release was controlled using the temperature-sensitive trans-genes UAS-*Shibire*^{ts} and UAS-TRPA1 coupled to a tyrosine hydroxylase Gal4 driver line (TH-Gal4). Determining alterations in visual behaviour was conducted with a visual choice maze that segregated fly populations according to their level of responsiveness to a moving grating, expressed as an Optomotor Index (OI). **Results:** Decreasing DA release, at any of the four developmental epochs, did not significantly alter visual responsiveness in male or female adult flies. However, increasing DA activity in male adult flies, during the third developmental epoch (5-7.5 days) significantly increased optomotor response (OI=1.24 ± 0.11, N=580 compared to both controls TH/+ (t = 7.024, p < 0.0001) and TRPA/+ (t = 6.994, p <0.0001), but not in females. Furthermore, increasing DA activity in male flies, during developmental epoch 7.5-10 days also significantly increased optomotor response (OI=1.35 ± 0.176, N=580), compared to both controls TH/+ (t = 6.96, p < 0.0001) and TRPA/+ (t = 5.710, p <0.0001), but not in females. **Conclusion:** We propose that increased optomotor responsiveness in flies reflects a failure in the ability to suppress a visual reflex. Our findings point to later stages in male fly brain development as a key epoch for establishing the dopaminergic circuitry necessary for attention-like processes such as stimulus suppression. Our results suggest that *Drosophila* may be an efficient model system to study underlying developmental or functional correlates observed in neurodevelopmental disorders that compromise attention, such as schizophrenia.

POS-TUE-003

OVEREXPRESSION OF TEN-M3 IN THE RETINA OF THE WALLABY M*ACROPUS EUGENII* ALTERS THE TOPOGRAPHY OF IPSILATERALLY-PROJECTING RETINAL AXONS

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Purpose: Ten-m3 is a transmembrane glycoprotein expressed in a decreasing ventrodorsal retinal gradient and a decreasing mediolateral gradient in the superior colliculus (SC) of the wallaby (*Macropus eugenii*). Here we examine its distribution in the dorsal lateral geniculate nucleus (dLGN) and assess the effects of its localised overexpression in the retina on the development of retinal projections. **Methods:** The expression of mRNA for Ten-m3 in the dLGN was investigated at early developmental stages by in situ hybridisation (n=4). Ten-m3 was overexpressed on postnatal day 18-22 by in vivo electroporation of a DNA construct encoding Ten-m3/green fluorescent protein (GFP) in localised regions of the retina (n=7). A control group was electroporated with a construct encoding GFP (n=5). Animals survived for 18-22 days before retinal projections were traced by an intravitreal injection of horseradish peroxidase. **Results:** In the SC there was an increase in ipsilateral projections to medial and rostral regions when Ten-m3 was overexpressed in the retina. In the dLGN there was a high dorsal to low ventral gradient of expression, corresponding topographically to its retinal gradient. The effect on ipsilateral projections was dependent on the retinal site of overexpression. An expansion was observed when Ten-m3 was overexpressed in ventronasal retina (n=3). Overexpression in dorsonasal retina produced no change (n=4). **Conclusion**: Overexpression of Ten-m3 alters the distribution of ipsilateral projections to the dLGN and SC. This complements data from Ten-m3 knockout mice and supports a key role for Ten-m3 in the control of ipsilateral retinal mapping in the brain.

POS-TUE-004

SEIZURE-RELATED GENE 6: A MODULATOR OF EXCITATORY SYNAPSE DEVELOPMENT

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Seziure-related gene 6 (Sez-6) is required for normal dendritic arbor development of cortical pyramidal neurons. While neurons lacking Sez-6 display excessive branching, these branches appear to be less able to support synapse development as excitatory synapses in the adult cortical neuropil are reduced by around 30%. The structure of Sez-6 also suggests a role in synapse biology. All three isoforms contain protein interaction domains (CUB and SCR) which are involved in neurotransmitter receptor trafficking and gating. **Purpose:** These experiments were aimed at investigating the role of Sez-6 in excitatory synapse development. **Methods:** Synaptogenesis and trafficking assays were performed in cultured neurons. Synapse development was monitored in Sez-6 null and control cortical neurons at a series of time-points (up to 18 days *in vitro*) by fixing and immunostaining for the vesicular glutamate transporter (VGLUT1; a presynaptic marker for cortical excitatory synapses) and post-synaptic density 95 (PSD-95). Colocalized regions (puncta size 0.8 - 4.0 μm^2) were scored. Antibody-feeding with the anti-Sez6 antibody was used to assess Sez-6 trafficking. **Results:** By immunoelectron microscopy, Sez-6 is detected in dendrites and dendritic spines. Trafficking experiments revealed that Sez-6 is trafficked from the cell-surface in recycling endosomes in an activity-enhanced manner. Excitatory synapses were observed to develop at a similarly low rate in Sez-6 wild-type (WT) and knockout (KO) neurons over the first week in culture. By 14 DIV, however, Sez-6 KO neurons exhibited significantly more synapses than WT neurons. Interestingly, this situation was reversed by 18 DIV. Neurons treated with secreted Sez-6 exhibited an increase in excitatory synapse number (50%, P<0.01) and colocalized puncta size (13%, P<0.01). **Conclusion:** Sez-6 is not required for the initial stages of excitatory synaptogenesis. In contrast, the maintenance of newly-formed synapses is enhanced by Sez-6, supporting a role in synapse maturation.

POS-TUE-005

THE COMPLEMENT ANAPHYLATOXIN RECEPTORS, C3AR AND CD88, DIRECT EMBRYONIC NEURAL PRECURSOR PROLIFERATION

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Purpose: The complement cascade of proteins is traditionally associated with the innate immune system. However, our group and others have recently shown novel roles for complement proteins, particularly in relation to neural development. This study demonstrates a role for the classical receptors to complement factors C3a and C5a (C3aR, CD88) in the proliferation of neural precursor cells (NPC) at the embryonic ventricle. **Methods:** Both receptors were localised, by immunofluorescence, to NPCs at the embryonic ventricle throughout embryonic development. To determine the functions of C3aR and CD88 on NPCs, C57BL/6J dams were administered 1mg/kg/day PMX53 (a selective CD88 antagonist), EP141 (a selective C3aR agonist) or vehicle control from days 12-14 of gestation (n=3/group). Embryos were dissected at E15 and the brain removed for analysis (>n=18/group). **Results:** Both qPCR and immunofluorescence for NPC markers showed a significant decrease in NPCs upon either C3aR stimulation, or CD88 inhibition, and these opposing roles for C3aR and CD88 are consistent with what is known of their functions in immunity. A corresponding increase in post-mitotic cells was also observed indicating that differentiation, not apoptosis, was responsible for the decrease in NPCs. Interestingly, this effect was unable to be demonstrated in C3aR or CD88 knockout mice, despite distinct behavioural differences, perhaps due to alternative mechanisms directing NPC proliferation/differentiation in these animals. **Summary** This study demonstrates that inhibition of CD88, or, alternatively stimulation of C3aR, favours differentiation of NPCs at the expense of proliferation. This is a novel and crucial role for the complement receptors in early mammalian brain development.

POS-TUE-006

MATERNAL DIETARY CREATINE SUPPLEMENTATION PROTECTS THE NEWBORN SPINY MOUSE BRAIN FROM INTRAPARTUM HYPOXIA

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Purpose: Using a model of intrapartum hypoxia in the precocial spiny mouse, we have previously shown that a maternal diet supplemented with 5% creatine monohydrate from mid-gestation improves survival and postnatal growth in offspring. The present study assessed the potential for maternal creatine supplementation to protect the fetal brain from the effects of intrapartum hypoxia. **Methods:** Pregnant spiny mice were fed a control (n=32) or 5% creatine-supplemented (n=24) diet from day 20 of gestation (term ~39 days). On day 38, pups were delivered by caesarean section (n=20 dams), or intrauterine hypoxia (n=36 dams) was induced by placing the excised uterus containing all fetuses in a saline bath for 7.5-8min, after which fetuses were expelled and resuscitation attempted by manual palpation of the chest. Surviving neonates were cross-fostered to a nursing dam for 24h. **Results:** Compared to control offspring, hypoxic offspring showed significantly increased lipid peroxidation in the brain, as measured by the amount of malondialdehyde ($p<0.05$), and significant increases in the number of cells expressing the pro-apoptotic protein Bax ($p<0.05$) and cytoplasmic cytochrome c ($p<0.05$) in the cortical subplate, thalamus and piriform cortex. When pregnant dams were fed the creatine supplemented diet, the increase in malondialdehyde, Bax and cytoplasmic cytochrome c was almost completely prevented such that they were not different from caesarean-delivered neonates. **Conclusions:** This study provides evidence for the neuroprotective capacity of creatine in the neonatal brain following severe hypoxia at birth. Further investigation into the long-term development and behavioural outcomes of such neonates is warranted.

POS-TUE-007

INTERNEURON SUBTYPE SPECIFICATION IS REGULATED BY SUPPRESSOR OF CYTOKINE SIGNALLING 2 (SOCS2)

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Gamma-aminobutyric acid (GABA)ergic interneurons play a vital role in modulating the activity of the cerebral cortex by inhibiting the activity of their neighbouring excitatory projection neurons. Disruption to interneuron function can directly contribute to neurological and mental health issues, such as schizophrenia, epilepsy and depression. Often such disruptions arise during development, with alterations to interneuron number, distribution or connectivity leading to functional neural impairment. However, little is known about the signalling mechanisms that regulate interneuron specification, migration or maturation. We have previously shown that the regulatory protein suppressor of cytokine signalling-2 (SOCS2) is a key player in interneuron development. SOCS2-overexpressing transgenic mice have large increases in numbers of calbindin and calretinin-expressing interneurons in the adult cortex. Whether this is due to altered interneuron specification during development or a result of the altered cortical environment of adult SOCS2 transgenic mice is unknown. During development SOCS2 is highly expressed in the ganglionic eminence of the ventral telencephalon, the primary source of cortical interneurons. SOCS2 is also highly expressed in the developing cortical plate, one of the main regions through which interneurons tangentially migrate to enter the cortex. To determine when the difference in cortical interneuron numbers in SOCS2 transgenic mice arises, we have compared interneuron subtype numbers over a developmental time course. No differences were observed between E13 and E17, suggesting that SOCS2 alters interneuron specification postnatally.

POS-TUE-008

INTRACELLULAR SIGNALLING PATHWAYS THAT REGULATE OLIGODENDROCYTE MYELINATION *IN VITRO*

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Purpose: Generation of the insulating myelin sheath is crucial for central nervous system (CNS) function. However, the nature of signals that regulate CNS myelination remain unclear. We have identified that the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) plays a key role in promoting myelination, and here we investigate its mechanism of action. **Methods:** *In vitro* myelination assays, consisting of co-cultures of dorsal root ganglia neurons and oligodendrocyte precursor cells (OPCs), were treated with BDNF, and either immunostained for MBP, or lysates generated and analysed by Western blot for expression of integral myelin proteins MAG and MBP. **Results:** We have identified that BDNF acts directly upon oligodendrocytes to promote myelination *in vitro* (n=3). In addition, our data strongly suggest that BDNF enhances CNS myelination via TrkB receptors, the cognate receceptor tyrosine kinase for BDNF, expressed on oligodendrocytes (n=3). To investigate the intracellular signalling pathways regulated by BDNF to promote myelination, we screened candidate downstream signalling pathways. We found that the activity of the MAPK/Erk signalling pathway positively correlated with BDNF-induced myelination *in vitro*, and that pharmacological inhibition of MAPK/Erk signalling blocked the promyelinating effects of BNDF (n=3). OPCs infected with constitutively active and dominant negative mutant constructs that regulate MAPK signalling indicate MAPK activation is critical for myelination *in vitro* (n=3). Furthermore, over-expression of Erk1 or Erk2 in OPCs suggests that Erk2, but not Erk1, is the MAPK that plays a key role in oligodendrocytes to enhance myelination (n=3). **Conclusion:** Together, our data have identified a novel role for MAPK signalling, through Erk2, within oligodendrocytes that regulate CNS myelination.

POS-TUE-009

CENTRAL INHIBITORY SIGNALLING MODULATES MORPHOLOGICAL DEVELOPMENT OF HYPOGLOSSAL MOTONEURONS

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Purpose: Inhibitory synaptic transmission modulates survival and development of motoneurons between E12 and P0. We used mice deficient in vesicular inhibitory amino acid transporter (VGAT) to examine the combined role of GABA and glycine in the morphology of hypoglossal motoneurons (XII MNs) at E18. **Methods:** XII MN counts were performed on hypothermia-anaesthetised E18 VGAT-deficient (KO) mice and wild-type (WT) litter mates using neuro-stereological methods. XII MNs in 300µm brainstem slices were prepared from E18 WT (*n*=11) and VGAT-KO (*n*=11) mice. XII MNs were then targeted by semi-loose seal Neurobiotin™ (NB) electroporation. Slices with NB-filled cells were fixed in 4% paraformaldehyde, incubated in Cy3-streptavidin and synaptophysin primary antibody, followed by secondary FITC conjugate. Mounted slices were imaged under a Zeiss LSM 510 Meta confocal microscope. **Results:** There were significantly fewer XII MNs at E18 compared with WT (-31%, P<0.05, *n*=6). Examination of individual XII MN morphology revealed the following in KO mice (*n*=11) compared to WT (*n*=11): i) significantly fewer dendro-dendritic dye-coupled cells (1.727 ± 0.6888 couplings per NB-filled cell) compared to (5.000 ± 1.027) in WT (P<0.05). ii) fewer primary dendrites (P<0.01), although these dendrites were longer in KO (447.3 ± 69.52µm) compared to (170.2 ± 20.00µm) in WT (P<0.01). iii) increased number of dendrites crossing to the contralateral brainstem for KO (1.300 ± 0.4955 crossings per NB-filled cell) compared to WT (0.09091 ± 0.09091 crossings, P<0.05). iv) a 6.5-fold increase in proportion of dendrites with spines (P<0.05). **Conclusion:** In the absence of inhibitory transmission, XII MN numbers are reduced. Surviving motoneurons appear to respond by reducing dye-coupling, increasing their dendritic length, contralateral projections and the number of spines.

POS-TUE-010

MYASTHENIC MUSK AUTOANTIBODIES REDUCE TYROSINE PHOSPHORYLATION OF ACHR AND DISPLACE ACH RECEPTOR AND RAPSYN FROM THE POSTSYNAPTIC SCAFFOLD

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Purpose: Recent studies have shown that autoantibodies against Muscle Specific Kinase (MuSK) from some myasthenia gravis (MG) patients cause disassembly of the postsynaptic AChR cluster leading to synaptic failure. MuSK is a postsynaptic receptor tyrosine kinase that is essential for formation of the neuromuscular junction (NMJ) during development. Activation of MuSK leads to tyrosine phosphorylation of the AChR βsubunit and stabilisation of AChR clusters. Here, we investigated mechanisms by which anti-MuSK antibodies causes disassembly of the NMJ. **Methods:** Mice were injected with control human IgG (45mg/day, 14 days) or IgG from an anti-MuSK-positive MG patient. Immunofluorescence and confocal microscopy on transverse sections of the tibialis anterior (TA) muscle was used to compare the intensity of postsynaptic membrane staining for AChR relative to rapsyn or β-dystroglycan. We also examined the intensity of postsynaptic staining for the tyrosine phosphorylated form of AChR β-subunit (Y390). **Results:** Mice injected with IgG from an anti-MuSK-positive MG patient 2 showed reduced postsynaptic staining for phosphorylated AChR βsubunit compared to control mice (n=3). Postsynaptic AChR and rapsyn were both reduced in anti-MuSK injected mice. In particular, the spatial correlation between rapsyn and β-dystroglycan staining was disrupted in anti-MuSK injected mice. **Conclusion:** βdystroglycan is thought to couple rapsyn (and the AChR) to the cytoskeleton. The current results suggest that Anti-MuSK IgG causes disassembly of AChR clusters by impairing the linkage between postsynaptic rapsyn-AChR complexes and dystroglycan-associated cytoskeleton.

POS-TUE-011

UNCOVERING THE MOLECULAR MECHANISMS OF THE TEN-M3 KO PHENOTYPE

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Purpose: Ten-m3 acts as an eye-specific axon guidance molecule for retinocollicular projections, and is critical for establishing connectivity within the visual pathway. Ten-m3 knockout (KO) mice have mapping errors of ipsilateral retinal projections to the thalamus and superior colliculus (SC), however the molecular mechanisms underlying these deficits are unknown. The aim of this study was to ascertain the molecular mechanisms underlying the Ten-m3 phenotype. **Methods:** Realtime PCR quantification was performed to assess whether mRNA expression of the well known topographic mapping molecules EphA and B and/or their ligands are altered in visual structures of Ten-m3 KOs. We also performed gene expression profiling on neonate retina and SC RNA of Ten-m3 KO and WT mice (n=3 per group) using the Affymetrix MoGene 1.0 ST microarray to investigate novel targets of Ten-m3. SAM and limma analysis was used to determine differentially expressed genes (Fold change ≥1.2, p ≤0.01), and GSEA to reveal functionally related gene groups and enriched biological themes. **Results:** Realtime PCR showed that EphA and EphB mRNA levels are altered in Ten-m3 KO thalamus, SC, and visual cortex. GSEA of differentially expressed genes suggests that Ten-m3 may regulate transcription, and implicates the Wnt signalling pathway as a potential mechanism for the KO phenotype. **Conclusion:** The intracellular domains of other Ten-m proteins have been shown to regulate transcription, and the results of this study intimate that Ten-m3 may regulate the transcription of axon guidance molecules in the visual system.

POS-TUE-012

ESTABLISHMENT OF AREAS IN THE PRIMATE VISUAL CORTEX: A ROLE FOR SEMAPHORIN 3A

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Purpose: To understand the role of the guidance molecule Semaphorin 3A (Sema3A) in the spatiotemporal development of visual cortical areas and their borders in the marmoset monkey (Callithrix jacchus). **Methods:** The expression profile of Sema3A and its receptor Neuropilin1 (Npn1) were established by immunolabelling and in situ hybridisation in the developing marmoset visual cortex, from embryonic day (ED) 88 to postnatal day (PD) 14 (n= 9). **Results:** At ED88, prior to completion of cortical lamination, Sema3A was homogenously expressed in the visual cortex, secreted by radial glia cells. After birth, as development proceeded, Sema3A expression pattern became bilaminar, and Sema3A was secreted by neurones located in layers 2 and 5. This transition from radial glia to neurones occurred in a specific spatiotemporal pattern, first in the primary visual cortex (V1) and the middle temporal area (MT), and subsequently in the second visual area (V2) and the other association areas. In addition the Sema3A receptor, Neuropilin1, was expressed as early as ED88, by neurones migrating out of the proliferative zone to the forming cortical layers. Later, Npn1 positive cells where located in layers 2 and 5. **Conclusion:** For the first time, we report an area specific expression pattern of Sema3A during the development of the nonhuman primate visual cortex. Our data suggest that the Sema3A/ Npn1 interaction could be involved in guiding neurones from the ventricular zone to layers 2 and 5 of the visual cortex in a spatiotemporal sequence that correlates with the sequential maturation of the visual areas in the marmoset monkey.

POS-TUE-013

METHODS TO ISOLATE AND CULTURE ADULT ZEBRAFISH CNS NEURONS: ROLE OF VARIOUS NEUROTROPHIC FACTORS

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Purpose: Central nervous system injury (CNS) can cause long lasting functional deficits that may have a devastating effect on an individual. The majority of neurons in the adult mammalian CNS have a very limited capacity for regeneration or axonal regrowth. However, the situation is quite different in lower vertebrates such as amphibians and teleost fish. These animals are capable of regenerating new neurons, regrowing injured axons and re-establishing functional connections. A deeper understanding of the factors involved in this repair and regeneration in a sub-mammalian species may give insights into how we may also repair the adult mammalian CNS. **Methods**: Disassociated neurons obtained from Adult zebrafish *(Danio Rerio*) brain were grown in BSF2 medium and kept in an incubator containing 1.5% carbon dioxide at 28.5°C. The cells were grown in either the presence or absence of various neurotrophic factors (BDNF, NGF, LIF, and NT3) at two different concentrations (1µl/ml and 5µl/ml) for a period of 7 or 14 days. There were at least six fish used in each possible experimental condition examined. Neurite growth was quantitatively assessed at the end of these periods using a stereological test grid and a one-way ANOVA procedure was used to test differences between experimental conditions. **Results and Conclusion**: We found that there was extensive neurite growth from adult zebrafish neurons under all conditions examined. Quantitative analysis revealed that there was a significantly ($P<0.05$) greater degree of neurite extension from neurons grown in the presence of LIF for 14 days compared to the other neurotrophic factors examined.

POS-TUE-014

IN SILICO MIRNA CANDIDATES REGULATING DOPAMINERGIC NEURON DEVELOPMENT

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Introduction Although the etiology of schizophrenia remains unknown, its epidemiology indicates it is developmental. Additionally, alterations in dopamine (DA) signaling remains a prominent finding in patients. With heritability estimates approaching 80%, genomic perturbation during the development of DA systems is a prime candidate for investigation. Recent findings from developmental animal models of this disorder indicate that important genes such as TH, DRD1, VMAT2, PTX3, Nurr 1, and p57/Kip2 are dysregulated, confirming that DA neuronal development and/or maturation may be perturbed in schizophrenia. As a first step in understanding the possible mechanisms behind this data we have conducted an in silico investigation of miRNAs as possible candidate regulators of these factors. **Methods** miRanda and 3'UTRdb were used to identify mRNA 3'UTR sequence and alternative initiation and splice variants for 20 major genes currently believed to be involved in DA neuron specification and maturation. A list of putatively interacting miRNA candidates was generated by complete alignment to the first 8 nucleotides of the 3'UTR mRNA target region. miRNAs with high mirSVR interaction scores were selected as candidates for further investigation. **Results** Of these 20 genes the 3'UTR targets with highest mirSVr scores were Nurr1, DRD1, PTX3, VMAT2, P57/Kip2 and PP1R1B, making them excellent candidates for further investigation. Hsa-miR-133b, -miR-7, -132, and -212 were the most highly represented miRNA candidates to regulate these genes. **Discussion** The ontogeny of these candidate miRNA and predicted gene targets will now be quantitatively investigated using miRNA arrays, and their interaction with 3'UTR mRNA targets assessed by biotin pull-down of the entire RISC complex. Hsa-miR-7, -132, and -212 have been previously reported to be dysregulated in schizophrenia and mir-133b has been validated as a negative regulator of DA neuron maturation and function, providing a putative link between abnormal dopamine neuron development and schizophrenia. This approach will then be applied in the DVD rodent model as abnormalities in DA ontogeny are implicated.

POS-TUE-015

VIRAL-MEDIATED INHIBITION OF ASTROCYTIC VESICULAR RELEASE

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Purpose: Astrocytes may modulate synaptic transmission via calcium-mediated vesicular neurotransmitter release. This process involves the VAMP/ SNARE complex, including synaptobrevin which is lysed and inactivated by the light chain of tetanus toxin (TeTxL). To examine the involvement of astrocytic vesicular release in neuronal function *in vivo*, we produced a replication-deficient lentivirus (Lv $Gfa(B)_3$TeTxL) expressing TeTxL under the control of an enhanced glial fibrillary acidic protein (GFAP) promoter ($Gfa(B)_3$). **Methods:** Microinjections of different concentrations of Lv$GFA(B)_3$TeTxL (4 x 100 nL injections) were made into the brain of anesthetized (ketamine 60 mg/kg/medetomidine 0.25 mg/kg im) Sprague Dawley rats (n=13). The rats recovered for 3-14 days before being deeply anesthetized and perfused via the heart with 4% paraformaldehyde. Expression of TeTxL and other cellular markers was examined by immunohistochemistry. **Results:** Expression of TeTxL was clearly observed at all time points in cellular processes without distinct somal expression. Injection of Lv$Gfa(B)_3$TeTxL, but not a similar control virus expressing green fluorescent protein, caused depletion of GFAP. At high virus concentrations, NeuN positive neurons near the injection site retracted dendritic processes and had a disc-like appearance. This was not evident at lower virus concentrations and shorter incubation times. The injection site showed macrophage (CD68) and lysosomal (CD63) infiltration and at 14 days evidence of cell death (SYTO15). **Conclusion:** Inhibition of astrocytic vesicular release has deleterious effects on both astrocytic and neuronal morphology and survival. With careful control of viral titres, this appears to be a promising approach for examining the role of astrocytic vesicular release in information processing by the brain *in vivo*.

POS-TUE-016

HIGH FREQUENCY STIMULATION OF THE INFRALIMBIC CORTEX MODULATES FOREBRAIN DOPAMINE TRANSIENTS

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Deep brain stimulation (DBS) has provided significant clinical benefit to people with movement disorders and has recently undergone trials for treatment-resistant neuropsychiatric indications, including treatment-resistant depression (TRD). A small number of trials of DBS for TRD have demonstrated the efficacy of the treatment with a large number of patients showing a sustained recovery from a severely debilitating form of depression. The neurophysiological mechanisms, however, remain to be determined. To address this issue, a series of experiments was conducted in urethane-anaesthetised male Sprague Dawley rats to determine the effect of DBS on transient dopamine efflux using fast-scan cyclic voltamettry at a carbon-fibre microelectrode. The effect of high and low frequency stimulation of the infralimbic cortex on transient dopamine neurotransmission in the core of the nucleus accumbens (NAcC) was examined. Clinically-effective, high frequency stimulation (HFS) of the infralimbic cortex (IL) of the Sprague Dawley rat was found to attenuate dopamine efflux in the NAcC, whereas clinically-ineffective low frequency stimulation (LFS) was found to have a negligible effect on dopamine efflux. HFS-induced attenuations of transient dopamine efflux were maintained a minimum of 2 hours post-DBS. This suggests that HFS may restore balance to cortical and limbic drive through the NAcC via modulation of this transient dopamine signal. This HFS-mediated enduring reduction of transient dopamine neurotransmission in the NAcC may be one mechanism of action responsible for the therapeutic effects of DBS in TRD.

POS-TUE-017

ALLEVIATION OF THE EFFECTS OF CHRONIC STRESS ON THE CA3-CA1 NEURONAL SYNAPSE IN THE RAT HIPPOCAMPUS

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Purpose: Chronic stress exposure has been shown to reduce/abolish long-term potentiation(LTP) at the hippocampal CA3-CA1 neuronal synapse, which is associated with changes in the dendritic morphology of these neurons. Recently, plant-derived odours have been shown to reduce the effects of chronic stress on the endocrine and sympathetic nervous systems. In the present study, we examined whether Praescent™ (cis-3-hexen-1-ol 0.03%, trans-2-hexenal 0.03%, alpha-pinene 0.015%, dissolved in tri-ethyl-citrate) can attenuate the effect of chronic stress at the CA3-CA1 neuronal synapse. **Methods:** 21day-old male Wistar rats were subjected to chronic restraint stress with/without concurrent Praescent™, for 4hours day/21days. Non-treated/Praescent™-treated control rats were also included. 24hrs following final treatment, animals were anaesthetized with sodium-pentobarbitone(60mg/k i.p.). Extracellular evoked field EPSPs at the CA3-CA1 neuronal synapse in hippocampal slices were recorded. LTP was induced by applying 3x1second(100Hz) trains, separated by 1-second intervals. Morphological examination of the CA3/CA1 pyramidal cells (Nissle and Golgi Cox stained) was performed on perfusion-fixed animals, using a Neurostereology-equipped microscope. **Results:** Electrophysiology recordings revealed a significant($p<0.0001$) 3-fold decrease in the magnitude of LTP in chronically-stressed rats(n=12) compared to control rats(n=12). This effect was attenuated($p<0.0001$) in rats stressed with concurrent Praescent™(n=12). Morphological analysis revealed a significant($p<0.0001$) reduction in apical-dendrite length/arborization of both CA1/CA3 pyramidal neurons in stressed-only rats(n=3) compared to control rats(n=3) and this was attenuated($p<0.0001$) in rats concurrently stressed with Praescent™(n=3). There was no significant change($p>0.05$) in neuronal number of CA3/CA1 pyramidal neurons across all treatment groups. **Conclusion:** Our results indicate that Praescent™ helps protect communication between dendritic synapses required for normal hippocampal LTP function by attenuating the effects of chronic stress at the morphological level.

POS-TUE-018

FUNCTIONAL IDENTIFICATION OF SPECIFIC VOLTAGE-GATED CALCIUM CHANNEL TYPES SUPPORTING NEUROTRANSMITTER RELEASE AT NEUROMUSCULAR SYNAPSES MISSING β2-LAMININ

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Purpose: At the neuromuscular junction (NMJ), transmitter release is initially dependent upon N-type voltage gated calcium channels (VGCCs) but later switches to P/Q type. β2-laminin has been shown in vitro to bind to P/Q channels suggesting a role in P/Q function and assembly close to transmitter release sites. Here, we employed specific N and P/Q VGCC toxins to functionally determine the importance of β2-laminin in the contribution made by N and P/Q VGCCs to NMJ transmission during postnatal development. **Methods:** ω-conotoxin GVIA and ω-agatoxin IVA target N and P/Q type VGCCs respectively. They were used to assess the relative contribution of N and P/Q to NMJ synaptic transmission in β2-laminin mutant and wild type mice. Phrenic nerves and diaphragms were dissected from P18 mutant and/or wild mice and mounted in an organ bath. Intracellular recordings of end plate potentials (EPPs) were taken in the absence of toxins and after the application of ω-conotoxin GVIA [1 x 10^{-8} M] (n=5) and ω-agatoxin IVA [5 x 10^{-9} M] (n=5) for both wild type and β2-laminin mutant mice. **Results:** At P18, mutants exposed to ω-agatoxin IVA showed a decrease in EPP amplitude of <40% while application of ω-conotoxin GVIA resulted in a decrease of >50% when compared to no toxin. Wild type showed a greater decrease (>60%) in the presence of ω-agatoxin IVA, and a lower decrease (<30%) after application of ω-conotoxin GVIA. **Conclusion:** Our present findings indicate a predominance of P/Q- type VGCCs in P18 wild type compared to β2-laminin mutants. The findings of this study suggested β2-laminin is necessary for the localisation of P/Q type channels close to active zones.

POS-TUE-019

SYNAPTIC DEGENERATION IN ALZHEIMER'S DISEASE

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Purpose: Synaptic dysfunction occurs early in Alzheimer's disease (AD) and is recognized as a primary pathologic target for AD treatment. Synapse degeneration or dysfunction contributes to clinical signs of dementia through altered neuronal communication and the degree of synaptic loss strongly correlates with cognitive impairment. The exact molecular mechanisms underlying synaptic degeneration are still relatively unclear; therefore, identifying abnormally expressed synaptic proteins in AD brain will help to elucidate those mechanisms and can lead to the identification of therapeutic targets that might slow AD progression. **Methods:** In the present study, synaptosomal fractions from post-mortem human brain tissue of AD (n = 6) and control (n = 6) were compared using 2D-differential in gel electrophoresis. AD pathology is region specific; human subjects can be highly variable in age, medication and other factors. Consequently, within each diseased state, hippocampus was compared with two relatively spared areas (motor and occipital cortices). Proteins exhibiting significant changes in their expression were identified (≥20% change, Newman-Keuls P-value <0.05) using either MALDI-TOF or ESI-QTOF mass spectrometry. **Results:** Using this proteomics approach, 70 synaptic proteins were found to be differentially regulated in hippocampus of AD brain when compared to controls. These proteins are involved regulating different cellular functions including energy metabolism, signal transduction, vesicle transport, structural and antioxidant function. **Conclusion:** This study showed that synaptic proteins in human AD brain are significantly different from those in control brain. Of the proteins identified in this study, a number have been studied in animal models, but only a few in human brain tissues. The functions regulated by these synaptic proteins with respect to AD will be discussed.

POS-TUE-020

SECRETED AMYLOID PRECURSOR PROTEIN-ALPHA ACTIVATES MAP KINASE AND CAM KINASE SIGNALLING PATHWAYS AT RAT CORTICAL SYNAPSES

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Aberrant processing of amyloid precursor protein (APP) in Alzheimer's disease leads to reduced secreted APP-alpha (sAPPα we have deduced pharmacologically that sAPPα upregulates protein) and concomitant rising levels of the neuropathological amyloid-β. sAPPα is a neuroprotective and neurotrophic protein which enhances memory mechanisms *in vivo* although the signalling pathways involved are unresolved. Previously synthesis at synapses via calcium-calmodulin dependent protein kinase (CaMK) and p42/44-mitogen activated protein kinase (MAPK) linked pathways. **Purpose:** To determine whether sAPPα treatment increases phosphorylation of MAPK and α-CaMKII at synapses. **Method:** Synapses (synaptoneurosomes) were prepared from the neocortex of young male Sprague-Dawley rats and treated with 10 nM recombinant human sAPPα or vehicle control (37°C). Phosphorylation status of p42-MAPK (Thr183/Tyr185) and α-CaMKII (Thr286) was assessed at several time-points by Western blot using phospho-specific antibodies. Phosphorylated protein was compared to total levels of the protein after normalising sAPPα treatment relative to control samples. **Results:** sAPPα treatment induced a significant increase in the proportion of phosphorylated p42-MAPK relative to total p42-MAPK (n=7, $p<0.05$). Likewise, sAPPα treatment stimulated an increase in phosphorylated α-CaMKII (n=5, $p<0.05$). In contrast, phosphorylation of eukaryotic initiation factor 4E and eukaryotic elongation factor 2 were not significantly altered. **Conclusion:** These results extend our previous findings by showing that sAPPα increases phosphorylation of both MAPK and CaMKII at sites important for kinase activity. These signalling molecules are both important for regulation of protein translation that may underlie maintenance of memory, therefore decreased sAPPα levels potentially contribute to memory dysfunction observed in Alzheimer's disease.

POS-TUE-021

THE LOCATION OF THE SLOW AFTERHYPERPOLARIZATION IN THE BLA

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In projection neurons of the basolateral amygdala (BLA), trains of action potentials (APs) are followed by a prolonged slow afterhyperpolarization (sAHP) that lasts several seconds and produces pronounced spike frequency adaptation. It is well established that the sAHP results from activation of a slow calcium-activated potassium current (sI_{AHP}); however, little is know about the channels that underlie the current. Both their cellular distribution and gating mechanism remain controversial. Here we investigate the subcellular distribution of these channels and whether their activation relies upon the calcium binding protein hippocalcin. Whole-cell patch-clamp recordings and high-speed calcium images were made from BLA projection neurons in rat brain slices. Channel location was probed by focally uncaging calcium using a UV laser in various cellular compartments. To test whether activation of the sI_{AHP} requires hippocalcin, neurons were transfected with an RNAi construct designed to knockdown hippocalcin. The sI_{AHP} was evoked by a depolarizing voltage-step that raised intracellular calcium throughout the neuron. Focally uncaging calcium in the soma evoked a current a fraction of the current evoked by the voltage-step (15±3%;p=0.0002;n=11), despite having a comparable calcium rise (108±12%;p=0.62;n=11). Similar magnitude currents were evoked by focally raising dendritic calcium (n=5). Comparison of the sI_{AHP} between neurons in transfected with the hippocalcin RNAi construct and untransfected control neurons is ongoing. The current evoked by focally raising dendritic calcium and the failure of the somatic calcium rises to reproduce the sI_{AHP}, indicate that this current is largely extra-somatic. The mechanism for the activation of the sAHP remains unclear.

POS-TUE-022

NICOTINE, TOTAL PARTICULATE MATTER OF CIGARETTE SMOKE AND THE DOPAMINE TRANSPORTER

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Purpose: Cigarette smoke is the leading cause of preventable illness worldwide and results in 5000 deaths per year in New Zealand alone. The majority of smoking addiction research focuses on nicotine treatment; however, there are over 4000 compounds present in cigarette smoke, some of which may contribute to addiction. The dopamine transporter (DAT) is important in regulating reward and has been shown to be modulated by nicotine. This study has investigated the effects of nicotine (0.35 and 3 mg/kg i.p.) and total particulate matter (TPM) from cigarette smoke on the expression and function of DAT. **Methods:** Dopamine uptake by DAT was measured in discrete brain regions of the rat using rotating disk electrode voltammetry. In addition, DAT protein and mRNA expression were measured using western blotting and quantatative RT-PCR. **Results:** TPM caused a significantly greater increase in DAT function than nicotine alone in the nucleus accumbens of *in vivo* treated animals ($p<0.01$). There has been no significant increase in DA uptake observed in the striatum of *in vivo* treated rats (n= 6) with nicotine or TPM, and no change in total protein in any brain region (n=3-6). There was; however, a significant decrease in DAT mRNA expression observed in response to nicotine, but not TPM, in the ventral tegmental area ($p<0.05$). **Conclusion:** Nicotine and TPM differentially modulate the function and mRNA expression of DAT. This work has increased our understanding of the non-nicotinic effects of cigarette smoke on DAT and may in future lead to the development of novel smoking cessation therapies.

POS-TUE-023

ARTIFICIAL INHIBITORY SYNAPSES

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$GABA_A$ receptors underlie the bulk of fast inhibition in the adult brain, and are targets for dozens of clinically important drugs. Development of new drugs targeting $GABA_A$ receptors is ongoing. *In vitro* experiments to this end usually involve exogenous application of GABA onto recombinant receptors, or neural cells in which there is a mixed population of receptors. We wished to explore the properties of defined $GABA_A$ receptor populations under realistic conditions of neurotransmitter release. We achieved this by expressing $GABA_A$ receptor subunits in AD-293 HEK cells, along with the post-synaptic adhesion molecule neuroligin 2A. When these HEK cells were incubated on top of cultured neurons from e18 rat hypothalamus for 24-48 hours, the neurons formed numerous synapses onto the HEK cells. These contacts were visualized as GAD65 and synaptophysin puncta on HEK cells. When the HEK cells were voltage clamped at room temperature, around 40% of cells had sufficient spontaneous post-synaptic currents for analysis (5 of 13 cells). On average, these events show a 10-90% rise time of 5.05±2.94ms and a decay time constant of 49.51±6.34ms. There was large inter-cell variability in event frequency (0.144±0.089Hz) and amplitude (110±77.6pA). Further experiments will use this technique to investigate the pharmacology of these synapses, and the importance of different subunits.

POS-TUE-024

CREATINE TRANSPORT IN NEURONS

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Creatine, an energy metabolite and well-known dietary supplement, is being tested as a neuroprotective agent in major clinical trials for Parkinson's and Huntington's disease. Despite this, how creatine is taken up and distributed within the brain remains poorly understood. Antibodies against the creatine transporter (CRT) were used to identify creatine uptake sites in rat and human brain. Strong immunostaining was found in specific sub-regions of the brain, such as pyramidal neurons of the motor cortex. In cultured rat embryonic hippocampal neurons, creatine transport activity increased as the neurons differentiated in culture. Most of the CRT was associated with dendrites. Neurons contained high levels of Na^+-dependent creatine transport activity (K_m = 45.5 μM; V_{max}, 1719 pmole creatine/ min/ mg protein) which was inhibited by competitive substrates of the CRT. The IC_{50} for the creatine precursor, guanidinoacetate was 712 μM, ~15 fold higher than the K_m for creatine. Incubation of neurons with 1 mM creatine resulted in the accumulation of high levels of creatine which affected the V_{max} but not the K_m for creatine transport. The rate of creatine release from neurons increased in the absence of Na^+ indicating the importance of the Na^+ electrochemical gradient for creatine retention. This is the first detailed study of the CRT in neurons and identifies primary cultures of rat hippocampal neurons as a good model for future studies of the CRT in relation to the effects of creatine on neuronal function and viability.

POS-TUE-025

ALTERATIONS IN POSTSYNAPTIC DENSITY PROTEINS WITH NEURODEGENERATIVE DISEASE IN THE HUMAN BRAIN

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PURPOSE: Glutamate receptors such as the N-Methyl-D-Aspartate receptors (NMDARs) and their bound Synapse Associated proteins (SAPs) are critical for normal brain function, including learning and memory, synapse development and plasticity. SAPs act as scaffolding molecules and are responsible for maintaining the structure of synapses, trafficking of receptors and activating signalling molecules. We hypothesise that these proteins could play an important role in the changes in synapse function that occur in response to neurodegenerative diseases such as Parkinson's Disease or Huntington's Disease. **METHODS:** We performed immunohistochemistry to visualise the expression of SAP97, PSD95 and the NR1 subunit of the NMDAR in human brain tissue from control, Huntington's and Parkinson's disease patients. **RESULTS:** We have found significant changes in the protein levels of SAP97 and PSD95 in the human hippocampus (SAP97, n = 6; PSD95, n = 3) and also in the striatum (SAP97, n = 3; PSD95, n = 2) in response to Parkinson's and Huntington's disease. In conjunction with changes in SAP expression levels, we have also shown an increase in NMDA receptor levels in Huntington's disease in the hippocampus (n = 5). We predict that the observed changes in the expression and localisation of the SAP proteins within the hippocampus of the human brain may underlie the loss of normal brain function in these diseases. **CONCLUSION:** These results provide insight into the altered subcellular mechanisms that could manifest into neurodegenerative diseases, providing potential for the development of more effective therapeutic strategies.

POS-TUE-026

THE EFFECTS OF STRONTIUM ON OUTWARD CURRENTS IN RAT HIPPOCAMPAL CA1 NEURONS

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Purpose: Strontium is a divalent cation that is a commonly used as a substitute for extracellular calcium to probe neurotransmitter release. It is generally assumed Sr only affects only pre-synaptic vesicular release. As transmembrane Ca flux affects several other processes including calcium-activated potassium conductances(I_{KCa}), we have investigated equimolar Sr substitution on neuronal outward currents. Method: Hippocampal slices from 3-7 week old Wistar rats were enzymatically treated and individual CA1 neurons (n=16) were obtained by mechanical dissociation. Outward currents were recorded using whole-cell voltage-clamp with a KF based pipette solution in electrodes. Sodium currents were blocked with tetrodotoxin and solutions rapidly applied via a 100 um diameter tube. Responses to depolarizing voltages commands were recorded before and after Sr treatment. Results: Sr substitution resulted in reversible reduction in amplitude of outward currents with kinetics and reversal potentials consistent with potassium conductances. Sr resulted in the abolition of a slowly activating, non-inactivating current, as well as rapidly activating and inactivating ("A-like") current. Computational modelling of this effect predicted increased excitability, consistent with previous current clamp studies in brain slice. Conclusion: Sr substitution for Ca in extracellular solution reduces outward current amplitude most likely through inhibition of calcium-activated potassium conductances. Care should be taken in interpretation of postsynaptic responses in the presence of Sr.

POS-TUE-027

MODULATION OF INHIBITORY GLYCINERGIC mIPSCs IN THE MOUSE MEDIAL NUCLEUS OF THE TRAPEZOID BODY

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Purpose Inhibitory glycinergic transmission in the medial nucleus of the trapezoid body (MNTB) is critical for sound localisation. Our previous experiments in the MNTB have revealed the existence of very large glycinergic mIPSCs which contribute to highly skewed amplitude distributions recorded from principal cells. We have used known modulators of neurotransmitter release in an attempt to resolve the mechanisms involved in generating these large events. **Methods** We recorded spontaneous glycinergic currents in mouse principal cells by whole cell patch clamp (in symmetrical chloride) at 35degC. **Results** Applying a hypertonic sucrose solution significantly increased both the average mIPSC amplitude (from 99pA to 196pA, n= 5, p= 0.009) and frequency (from 7Hz to 30Hz n =5, p =0.002). The calcium ionophore, ionomycin (10μM) significantly increased the mean frequency of events (from 2.2Hz to 12.2Hz, n =7, p =0.02) without a corresponding change in mean amplitudes (from 125pA to 136pA, p = 0.62). Introducing 100μM gabazine (a low affinity antagonist at glycine receptors) resulted in a uniform reduction in mean amplitudes (from 192pA to 106pA, n=5, p = 0.018) without a corresponding change in the shape of the amplitude distributions. Blocking glycine reuptake with 20μM ORG24598 significantly increased the frequency of events (from 1.9Hz to 6.9Hz, n=7, p=0.001) but did not alter mean amplitudes (177pA before, to 200pA, p=0.32).**Conclusion** These results are most consistent with the observed large range in mIPSC amplitude being due to site to site variability in mean mIPSC amplitude. This would result in large stochastic amplitude fluctuations in evoked inhibitory transmission, which is likely to play an important role in signal processing in the auditory brainstem.

POS-TUE-028

VOLTAGE-DEPENDENT AMPLIFICATION AND ATTENUATION OF THE CHOLINERGIC RESPONSE BY KV7 CHANNELS

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Purpose: Acetylcholine (ACh) plays an important role in sensory processing tasks such as attentional modulation. Phasic ACh release regulates the tuning properties of cortical neurons by increasing firing rates via a muscarinic (M_1) acetylcholine receptor-induced depolarisation. It is generally thought that the underlying ionic mechanism is a closure of the muscarine-sensitive Kv7 channel (M-current) but the evidence for this modulation in the cortex is scarce. **Methods:** Whole-cell and perforated patch-clamp recordings were made from layer 5 pyramidal neurons in rat cortical slices. Focal application of ACh (100 μM; 400 ms) was achieved by placing application pipettes ~20 μm from the soma. **Results:** From resting potential ACh produced a slow (~4 s time-to-peak) M_1–mediated depolarisation with average peak amplitude of 2.6 mV (n = 31). The depolarisation increased to 3.7 mV after blocking Kv7 channels (10 μM XE-991; n = 22, p < 0.01), indicating that M_1 mediated Kv7 channel closure is not necessary for the ACh-induced depolarisation. These data could be explained by the increased input resistance caused by Kv7 blockade, which closely correlated with the amplification of ACh-induced depolarization (r^2 = 0.63). To test the voltage range more extensively ACh was applied in the presence of TTX (0.5 μM) and at gradually depolarised membrane potentials (up to 0 mV). The results showed that ACh responses were attenuated in XE-991 only at potentials > –40 mV (n = 5). **Conclusion:** Kv7 channels have a complex dual role in the ACh response; at subthreshold potentials they curtail the ACh response but at suprathreshold potentials, via M_1 receptor mediated-inhibition, Kv7 channels mediate the cholinergic response properties.

POS-TUE-029

NEUROANATOMICAL AND NEUROCHEMICAL FEATURES OF PARVALBUMIN-EXPRESSING NEURONS IN THE MOUSE SPINAL DORSAL HORN

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We have previously shown that Parvalbumin (PV) expressing neurons in the mouse spinal cord dorsal horn exhibit distinct functional properties including high frequency tonic AP discharge, a high incidence of Ih currents, and relatively low excitatory synaptic drive. **Purpose:** To further characterize this population by identifying i) the morphology and neurochemical features of PV-expressing neurons in the dorsal horn; ii) their sources of afferent input; and iii) their main postsynaptic targets. **Methods**: Transverse or sagittal sections from lumbar segments of adult ICR mice (n = 3) were first incubated in a cocktail of primary antibodies and then species-specific secondary antibodies tagged with fluorescent markers to reveal labelling for PV, vesicular GABA transporter (VGAT), vesicular glutamate transporters 1 and 3 (VGLUT1, VGLUT3), IB4, CGRP and PKCγ. Confocal image stacks were collected from representative sections in each animal to determine the morphology of PV-ir neurons, their main afferent input, and the relationship of PV-ir axons with afferent terminals. **Results:** The main concentration of PV-ir neurons occurred in lamina III with PV-ir axons ramifying mainly in lamina IIi. PV-ir neurons often displayed islet cell-like morphology, with large somas and elongated dendritic arbors that extended in the rostro-caudal plane. The main primary afferent input to these neurons was from VGLUT1-ir terminals (35.2 per neuron; range 24-42) and IB4-containing C-fibres (29.8 per neuron, range 17-36), whereas inputs from peptidergic afferents were rare (3.6 per neuron, range 1-7). Inhibitory (VGAT-ir) PV-ir axon terminals targeted VGLUT1-ir axon terminals in lamina IIi and III almost exclusively forming presumptive axo-axonic synapses. **Conclusions:** These results show that PV-containing neurons in the mouse spinal dorsal horn include islet cells, receive strong afferent input from Aβ and non-peptidergic C-fibres, and mediate presynaptic control over low threshold mechanoreceptor and down-hair afferents.

POS-TUE-030

RTMS INCREASES INTRACELLULAR CALCIUM IN CULTURED CORTICAL CELLS

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Purpose: Stimulation of brain tissue by administration of repetitive transcranial magnetic stimulation (rTMS) benefits many neurological and psychiatric disorders. The effects of rTMS are generally assumed to involve the electrical properties of neural cells, but it is not clear how neural activity is translated into long-term changes in circuitry. Here we have used mixed cultures from neonatal mouse cortex to study the impact of rTMS on intracellular calcium levels. Methods: C57Bl/6j mice were euthanased at P1 and mixed neuronal/glial cultures were prepared from cortex and grown for 10 days prior to experimentation. Intracellular calcium was assessed by monitoring alterations in Fura-2 340/380nm ratiometric fluorescence in response to rTMS applied for 10 minutes using a custom-built coil (10mT field intensity; 10Hz frequency; modified *e-cell*™, Global Energy Medicine, Perth, Australia)). The ratiometric values measured for the final 3 minutes of the 10 minute stimulation period were averaged and reported as a percentage from the pre-stimulation baseline average. Results: From three separate experiments, a total of 10 control and 10 rTMS cells were analysed. In all of the cells that received rTMS, there was a significant increase in Fura-2 signal (6.5 ± 0.9%; $p<0.05$). In 3 of the 10 cells, a further increase in Fura-2 was detected after stimulation was ceased. Conclusions: rTMS results in alterations in intracellular calcium homeostasis. Future studies will examine the effect of varying frequency and intensity on calcium influx and relate these changes to cell survival and neurite outgrowth.

POS-TUE-031

MUCOSAL APPLICATION OF L-GLUTAMATE EVOKES LOCAL INHIBITORY REFLEXES IN ISOLATED GUINEA-PIG JEJUNUM

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Purpose. Umami is a unique taste, distinct from sweet, salty, sour, and bitter. Recently, receptors and molecules involved in umami taste mechanisms have been identified in the intestinal mucosa. L-glutamate elicits the umami taste. The aim of this study was to examine whether mucosal application of L-glutamate activates local neural reflexes similar to those evoked by the aromatic amino acids L-phenylalanine and L-tryptophan. **Methods.** Segments of jejunum were dissected to allow access to the circular muscle on half of the preparation, leaving the mucosa intact on the circumferentially adjacent half. Circular muscle cells were impaled close to the intact mucosa and L-glutamate (30 mM) or single electrical stimuli (ES) were applied to the mucosa 1-2mm circumferential to the impalement site. **Results.** L-glutamate evoked local inhibitory junction potentials (IJPs) in the circular muscle similar to those seen evoked by L-phenylalanine or L-tryptophan (amplitude 3-10 mV, latency 150-450 ms, N>20). PPADS (10 μM) and tropisetron (10 μM) significantly reduced these IJPs by 75% (N=5, P<0.001) and 10% (N=8, P=0.02) respectively but did not affect electrically stimulated IJPs. DNQX (10 μM, N=6) or CNQX (10 μM, N=4) reduced the IJPs evoked by L-glutamate by 80-90-% (both P<0.001), but also reduced electrically stimulated IJPs by 30-40% (both P<0.001) suggesting non-specific action at inhibitory neuromuscular junctions. Neither BAY367260 (mGluR5 antagonist, 10 μM) nor 2APV (30 μM) affected IJPs evoked by L-Glutamate or ES. **Conclusions.** Mucosal application of L-glutamate activates local inhibitory reflexes via the release of ATP and 5-HT from the mucosa, which acts at P2X receptors or 5-HT_3 and/or 5-HT_4 respectively. Other receptors involved remain to be determined and may include AMPA, but not NMDA or mGluR5 receptors.

POS-TUE-032

PHOSPHORYLATION OF α3 GLYCINE RECEPTORS PRODUCES AN EXTRACELLULAR CONFORMATIONAL CHANGE

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The glycine receptor (GlyR) extracellular M2-M3 linker domain is important for initiating channel activation. We previously employed voltage clamp fluorometry (VCF) to monitor conformational changes in the α1 GlyR M2-M3 domain. VCF involves tethering a rhodamine to introduced cysteines and monitoring fluorescence and current changes during activation. Here we sought to determine whether the α3 subunits responded similarly to α1 subunits during activation. Oocytes were surgically removed from anaesthetized frogs by procedures approved by the University of QLD Animal Ethics Committee. GlyR mRNA was injected into Xenopus oocytes and GlyR function was studied using VCF. During glycine activation, the α3-R19'C GlyR exhibited a dramatically decreased glycine sensitivity (EC50~12 mM; all results averaged from > 5 cells). Fluorescence of the label attached to α3-R19'C GlyR increased by ~5% and the glycine fluorescence and current dose-responses overlapped. Although the structures of α1 and α3 are identical in and around the M2-M3 domain, relative to the α1-R19'C, the α3-R19'C fluorescence was much smaller compared (~20%) and much slower to return to baseline after glycine removal. To isolate the domain responsible for these differences, we generated a series of eight chimeras and found the intracellular M3-M4 domain to be responsible for this difference. An α3 GlyR-specific PKA consensus sequence (S346) has been identified in this domain. Treatment of α3-R19'C oocytes with forskolin effected an inhibition of glycine-activated florescence. Our results suggest that phosphorylation of the M3-M4 domain induces conformational changes in the M2-M3 loop. This provides a structural basis for understanding how phosphorylation can change current magnitude and desensitization properties.

POS-TUE-033

DEEP BRAIN STIMULATION OF THE NUCLEUS ACCUMBENS ATTENUATES TRANSIENT DOPAMINE NEUROTRANSMISSION

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High frequency deep brain stimulation (HF DBS) of the nucleus accumbens (NAc) is a neuromodulation technique currently being trialled for refractory psychiatric disorders. The NAc is a key region of the mesocorticolimbic dopamine (DA) system. NAc DA mediates cortical and limbic neuronal information flow through the basal ganglia and dysregulation of information processing in this region is implicated in several psychiatric conditions. NAc HF DBS has demonstrated therapeutic efficacy for treatment of refractory obsessive-compulsive disorders (OCD), depression and Tourette's syndrome, yet the therapeutic mechanisms of DBS remain to be determined. This study aimed to investigate how NAc DBS modulates transient DA release in this region. To achieve this, the effects of NAc HF (130Hz) and low frequency (LF) (10Hz) DBS (90 minutes) on VTA-evoked transient DA transmission were determined in urethane-anesthetised rats. Transient DA release was monitored utilising real-time voltammetry. HF NAc DBS induced immediate suppressive effects on transient DA release that continued for 2 hours post-DBS. In contrast, LF DBS had no effect on transient DA. The results have important clinical implications suggesting that the therapeutic effects of NAc DBS could be attributable, in part, to attenuation of DA transients.

POS-TUE-034

ALTERED ELECTROPHYSIOLOGICAL PROPERTIES OF PURKINJE CELLS IN THE DYSTROPHIN-DEFICIENT MDX DYSTROPHIC MOUSE

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In cerebellar Purkinje cells dystrophin is co-localized at the postsynaptic membrane with GABAA receptors. In young mdx mice, where full-length dystrophin is absent, there is a 50% reduction of GABAA receptor α1 and α2 subunits in Purkinje cells. Interestingly, while these GABAA receptor subunits decreased with age in WT, no such change was seen in mdx. Here, we investigate the electrophysiological effect of the reduction of GABAA receptors in cerebellar mdx Purkinje cells. We also investigate if the functional deficits in LTD, which we have previously reported in mdx Purkinje cells, are ameliorated in aged mice. All experiments were performed in cerebellar slices from mdx and age-matched WT mice. The RMP was significantly more depolarised -61mv +/- 2 (n=13) in aged mdx cells compared to aged-matched WT -70mv +/- 2 (n=9), which is consistent with an altered intracellular ionic balance. During LTD induction, WT eEPSPs initial slope displayed significantly greater depression at early but not late phases compared to aged mdx (early phase Wt 71+/- 4.% (n=5); mdx 59 +/- 3% (n=6) p=0.0302 : late phase WT 63 +/- 4% (n=5); mdx 56 +/- 2% (n=6) p=0.0844). This is in contrast to our previous report in young mdx where both phases of LTD were blunted. Using non stationary noise analysis, we found a significant difference in the number of receptors at GABAergic synapses in young mdx mice (38.38 ± 2.95; n=14) compared to WT (53.03 ± 4.11; n=12) (p= 0.01). If similar changes occur in DMD, it may contribute to the motor, behavioural and cognitive impairment apparent in many boys with DMD.

POS-TUE-035

POTENTIAL BLOCKADE OF HETEROMERIC NICOTINIC RECEPTORS BY PINNATOXINS E AND F

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Purpose: The cyclic imine toxins, gymnodimine and spirolides, have been found to be potent antagonists of both muscle type and neuronal nicotinic acetylcholine receptors, causing death within minutes by respiratory depression. This toxicity is shared by the novel cyclic imine pinnatoxins E and F ($PnTx_{E/F}$), produced by marine dinoflagellates and recently isolated from New Zealand shellfish. However, there is currently little data available regarding the mechanism of action for any of the pinnatoxins, and no data at all on $PnTx_{E/F}$. The aim of the current study was to investigate potential $PnTx_{E/F}$ antagonism of nicotinic acetylcholine receptors using two *in vitro* tissue preparations. **Methods:** Phrenic nerve-evoked compound muscle action potentials (CMAPs) were recorded from hemidiaphragm to assess $PnTx_{E/F}$ effect on heteromeric neuromuscular nicotinic receptors. $PnTx_{E/F}$ effects on α7 homomeric neuronal nicotinic receptors were investigated by recording gamma oscillations in response to *in vitro* tetanic stimulation of hippocampal region CA1. **Results:** Crude extracts of $PnTx_{E/F}$ had no effect on hippocampal gamma oscillations (500 nM; n=4 slices), but caused a decrease in amplitude of the hemidiaphragm CMAPs to 38 ± 14% and 29 ± 18% at concentrations of 500 nM (n=5) and 3 µM (n=4), respectively (P<0.05 each). Pure pinnatoxin F also caused a dose-dependent reduction in CMAP amplitude, with decreases to 31 ± 15% and 25 ± 11% of baseline at 260 nM (n=4) and 520 nM (n=4), respectively (P<0.01 each). **Conclusion:** These results show that $PnTx_{E/F}$ blocks neuromuscular transmission and suggest that observed *in vivo* muscle paralysis by pinnatoxin is due to selective antagonism of muscle type nicotinic acetylcholine receptors.

POS-TUE-036

ANGIOTENSIN II TYPE 1A RECEPTOR MOTILITY AND FILOPODIA DYNAMICS

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Purpose: The dynamics of G protein-coupled receptors (GPCRs) within a highly motile cell membrane are not understood. Therefore, we have developed an experimental approach to measure membrane motility and GPCR motility in live cells, during application of receptor agonists and an inhibitor of actin polymerisation, cytochalasin-B. **Methods:** We used Chinese Hamster Ovary (CHO) cells expressing angiotensin II type 1a receptor (AT1AR) tagged with enhanced green fluorescent protein (eGFP) as a GPCR model system. Cells were imaged with a Leica SP5 confocal microscope equipped with a high-speed resonant scanner and avalanche photo diode detectors. Series of 50 images of live CHO-AT1AR-eGFP cells were captured at 20-25 frames per second, t=0, 10, and 20 min after addition of fluorescently labelled AT1AR agonist, angiotensin II-AlexaFluor647 (AngII-A647;100nM) or cytochalasin-B (0.5-5µM). Images were collated, processed and analysed with ImageJ. **Results:** In the absence of agonist, a surprising amount of cell membrane motility, especially filopodia generation and movement, and constitutive internalisation of the receptor occurred. In the presence of agonist, the high level of membrane motility remained during internalisation of ligand-bound receptors. Treatment with cytochalasin-B caused dose- and time-dependent reorganisation of actin structures, loss of membrane motility, and inhibition of receptor-mediated endocytosis. **Conclusions:** These observations suggest that the analysis of agonist-receptor interactions, at least in cell lines, will be significantly confounded by the consequences of membrane motility. Furthermore, GPCR activation is likely to be influenced by the state of the actin cytoskeleton associated with the membrane. The role of such interactions in neuronal excitability and plasticity remain to be determined.

POS-TUE-037

VESICULAR GLUTAMATE AND GABA TRANSPORTERS IN HUMAN AUTOPSY BRAIN TISSUE

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Purpose: Alzheimer's disease is the most common form of dementia. Synaptic loss and changes in synaptic proteins correlate closely with disease progression and severity. Recent studies suggest that the vesicular glutamate transporter proteins are affected early in AD pathogenesis and that this impacts on cognitive function. mRNA expression of the vesicular glutamate transporters VGLUT1 and VGLUT2, and the vesicular inhibitory amino acid transporter (VIAAT), was measured in human autopsy brain tissue from AD and control cases. **Methods:** A real-time RT-PCR assay was developed to quantify the transcripts of VGLUT1, VGLUT2, and VIAAT in *post mortem* brain tissue. Three regions were examined in each case - hippocampus, inferior temporal cortex (ITC) and occipital cortex. **Results:** The sensitivity and specificity of the assay were confirmed using neurologically normal human brain. VGLUT1 and VGLUT2 were highest in the ITC and lowest in the hippocampus. VGLUT1 was the most abundant glutamate transporter in all areas, consistent with previous research. VIAAT expression was comparable in the ITC and occipital cortex, but lower in the hippocampus. VIAAT expression was low in all areas, which is surprising due to the reported abundance of GABAergic neurons in the cerebral cortex. In a pilot study, no significant differences were observed between AD (n=5) and control (n=5) cases, although a pattern of reduced expression was observed in affected areas of AD cases *cf* controls. **Conclusion:** mRNA expression of vesicular amino acid transporters can be quantified in human autopsy brain tissue. This is the first study to investigate the expression of VIAAT mRNA, the only known vesicular transporter for GABA and glycine, in the human brain.

POS-TUE-038

REQUIREMENT FOR NITRIC OXIDE AND PROTEIN SYNTHESIS IN PRESYNAPTIC COMPONENTS OF PERSISTENT HIPPOCAMPAL LTP

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Long-term potentiation (LTP) is an important process underlying learning and memory in the brain. At CA3-CA1 synapses in the hippocampus, three discrete forms of LTP (LTP1, 2 and 3) can be differentiated on the basis of maintenance and induction mechanisms. However, the relative roles of expression mechanisms in LTP1, 2 and 3 are unknown. We investigated enhanced neurotransmitter release in the expression of LTP1, 2 and 3 by measuring destaining of FM1-43 from potentiated CA3 terminals in 400µm brain slices from male Wistar rats (7-8 weeks). No difference in vesicle turnover rate was observed for LTP1 at 60 min or 120 min following induction by 1 train of theta-burst stimulation (1TBS). An increase in release was found for LTP2 only at 120 min after induction by 4TBS ($n=6$; $p<0.05$), and for LTP3 at both time points after induction by 8TBS (60 min, $n=14$; 120 min, $n=10$; $p<0.05$). Inhibition of nitric oxide (NO) signalling with L-NAME blocked both LTP2 and LTP3 maintenance and the associated enhanced release (LTP2, $n=5$, $p<0.05$; LTP3, $n=4$, $p<0.05$). Inhibition of protein synthesis with anisomycin blocked both LTP2 maintenance ($n=4$, $p<0.05$) and the associated enhanced exocytosis ($n=3$, $p<0.05$), whereas the transcription inhibitor Actinomycin-D had no effect. LTP3 maintenance was dependent on both protein synthesis ($n=4$, $p<0.05$) and transcription ($n=6$, $p<0.05$), however the enhanced release was dependent only on protein synthesis ($n=4$, $p<0.05$). These results demonstrate that more durable forms of LTP involve a NO-dependent enhancement of transmitter release that is dependent on *de novo* protein synthesis but not gene transcription.

POS-TUE-039

SYNAPTIC TRANSMISSION IN THE MEDIAL NUCLEUS OF THE AMYGDALA

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BACKGROUND: The medial nucleus of the amygdala receives chemosensory inputs from the main and accessory olfactory systems and plays a key role in defensive and reproductive behaviour. However, synaptic transmission in this nucleus remains poorly understood. **METHODS:** We have studied the synaptic responses of neurons in the posterioventral nucleus of the MeA (MePV) in adult male GAD67-eGFP knock-in mice using whole cell patch-clamp recordings in acute brain slices. Synaptic responses were evoked by stimulating the afferents locally, next to the soma, and/or by stimulating the olfactory axons within the MeA molecular layer. Cells were filled with biocytin during recording and later visualized using immunohistochemistry and Alexa Fluor 568 labelling. RESULTS: Synaptic stimulation in both GFP+ (n=21) and GFP- cells (n=53) evoked polysynaptic GABAA-mediated inhibitory responses and monosynaptic glutamatergic responses that were mediated by non-rectifying AMPA and NR2B-containing NMDA receptors. Synaptic currents evoked by the stimulation of the olfactory axons in the external molecular layer showed slower decay time constant as compared to those evoked by local stimulation (11.26±8.1 vs. 3.1±1.5, $n=15$; $p<0.01$) as well as slower rise times (1.9±1.1 vs. 1.1±0.5; $p<0.01$) suggesting that olfactory synapses are electrotonically more distant. The paired-pulse ratio at the olfactory synapses was mainly facilitating (1.3±0.3, $n=15$), whereas the local input synapses were mostly depressing (0.9±0.3, $n=15$; $p<0.01$). Finally, The morphological study of the recorded cells shows that their dendrites extend to the molecular layer forming distal dendritic tufts. **CONCLUSION:** We propose that the MePV neurons receive converging inputs at separate locations on their dendritic tree, with the olfactory inputs synapsing mainly at the distal dendrites and the intraamygdala afferents synapsing at the proximal dendrites.

POS-TUE-040

SPATIAL EXTENT OF RETINAL ACTIVATION USING EPIRETINAL HEX ELECTRODES

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Purpose: The aim of this study was to characterise retinal ganglion cell activation following epiretinal electrical stimulation with hexagonally-arranged bipolar (hex) electrodes, to evaluate the feasibility of this electrode arrangement for future retinal implant designs. **Method:** *In vitro* experiments were performed on rabbit retinal preparations. Single-unit recordings were obtained from ganglion axons using extracellular tungsten microelectrodes. The cell was stimulated along its axonal path with 125 µm diameter platinum hex electrodes, where the centre electrode was the current source and the six surrounding electrodes being returns. 100 and 300 µs/phase anodic-first biphasic pulses were delivered, and cell responses to 10 consecutive pulses were recorded. This procedure was repeated for a range of stimulus amplitudes, with threshold defined as the current amplitude which elicited a cell response 50% of the time. To estimate the threshold as a function of distance from the stimulating electrodes, the electrodes were moved perpendicular to the axonal path. **Results:** Thresholds for axonal activation increased up to 10 fold at a distance of 150 µm from the centre electrode (n=12 cells). The threshold was well-described by a power function with lateral distance, except immediately beneath the electrode disk where the threshold remained constant. With 100 and 300 µs/phase biphasic pulses, power function exponents were 1.84 and 1.63 respectively. **Conclusion:** Ganglion cell axonal activation thresholds increased markedly with distance from the hex centre, suggesting that localised retinal activation is achieved with this electrode arrangement. Activation tended to be even more localised with briefer stimuli.

POS-TUE-041

QUANTIFICATION OF GLYCINE TRANSPORTER-2 DISTRIBUTION SURROUNDING BUSHY CELLS IN THE MOUSE COCHLEAR NUCLEUS

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Purpose: Central auditory processing for sound localisation is initiated in the cochlear nucleus at giant excitatory synapses onto two bushy cell types. Bushy cells also receive significant amounts of glycinergic inhibitory inputs, the role of which is still unknown. This study aims to quantify and compare the glycine transporter-2 (GLYT2) distribution on the inputs to spherical (SBC) and globular bushy cells (GBC). **Methods:** Immunohistochemistry was used to locate GLYT2, while bushy cell types were identified based on their somatic profiles using propidium iodide. Quantification of GLYT2-immunopositive presynaptic terminals was performed in 50 µm parasagittal sections of the cochlear nucleus using fluorescence microscopy and ImageJ software for analysis. **Results:** Preliminary results in normal hearing mice (n=2) demonstrate no significant difference between the numbers of GLYT2-immunopositive puncta directly apposed to SBC somata (8.3 ± 0.8; n=6) versus those apposed to GBC somata (7.5 ± 0.4; n=6; $P>0.05$). The total area of the GLYT2-immunopositive puncta surrounding SBC somata (39.0 ± 7.2) was also similar to the total area surrounding GBC somata (52.2 ± 9.7; $P>0.05$). **Conclusions:** Future studies will compare these findings with GLYT2 immunolabelling and electrophysiological recordings in congenitally deaf mice (*Shaker-2*). These data will lay a foundation to explore how deafness perturbs the balance of excitation and inhibition.

POS-TUE-042

NON-LINEAR RADIAL GAIN OF OPTOKINETIC NYSTAGMUS DIFFERENTIATES TWO NEURAL SYSTEMS

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Optokinetic Nystagmus (OKN) is a stereotyped repetitive movement of the eyes that is evoked in response to global motion of the visual field. It consists of slow stimulus tracking phases alternating with fast saccadic returns which is observed in nearly all vertebrates. This reflex is generated in large part by the accessory optic system which has been shown in rabbit to be driven by a unique type of retinal ganglion cell (RGC), the ON- direction selective (ON-DS) ganglion cell. The population of ON-DS ganglion cells are tuned to 3 different directions. It is unknown, however, how the visual system integrates these three preferred axes to generate OKN. We have therefore examined the gain of both reflexive and voluntary OKN along different radial axes in humans (n = 12). Random dot stimuli (100 dots drifting at 30°/sec in 15° intervals) were generated on a video monitor (27.4 x 27.4°) using Vision Egg software. Eye movements were captured with a video camera, quantified using a custom program written in R and analysed with Labview. In reflexive OKN, vertical and horizontal eye movements were similar whereas nearly all other angles had reduced gain. In contrast, voluntary OKN had equivalent gain for all directions. These data suggest that the generation of OKN under the two instructional conditions must be different and that the integration of retinal input to the accessory optic system is non-linear, whereas the retinal input driving voluntary OKN is linear. These data quite clearly demonstrate that two neural systems are required for the generation of OKN in humans.

POS-TUE-043

NEUROKININ 1 (NK1) RECEPTOR IS INTERNALISED IN SACRAL DORSAL HORN AND AUTONOMIC PREGANGLIONIC NEURONS IN RESPONSE TO ACUTE AND CHRONIC BLADDER INFLAMMATION

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Purpose: After acute noxious stimulation, the neurokinin 1 (NK1) receptor internalises in specific dorsal horn neurons of the spinal cord. This has been used as a measure of substance P (SP) release and peptidergic transmission. Our aim was to utilise NK1 receptor internalisation to identify individual autonomic preganglionic and dorsal horn neurons that are activated by noxious stimulation of bladder nociceptors and by bladder inflammation. **Methods:** Studies were performed in adult female Sprague-Dawley rats (4 per group). To activate bladder nociceptors, capsaicin (0.03%,100µl) was instilled into the lumen for 10 min (ketamine/xylazine anaesthesia), followed by intra-cardiac perfusion with fixative. Lower urinary tract inflammation was induced by cyclophosphamide (CYP; 75 mg/kg i.p., isoflurane anaesthesia), and tissues removed after 48 h ("acute") or 10 days (following further CYP injections at 3 and 7 days; "chronic"). Spinal cord sections were processed for immunofluorescence and confocal microscopy. **Results:** The majority (~80%) of sacral IML neurons expressed NK1 and this proportion was unaffected by treatment. Quantification of NK1+ endosomes revealed activation of superficial dorsal horn and IML neurons in all treatment groups, with comparable internalisation in each location and treatment (~10 endosomes per neuron, c.f. none in controls). **Conclusion:** This is the first study to visualise the internalisation of NK1 receptor in individual dorsal horn and preganglionic neurons in response to bladder inflammation. Chronic bladder pain is accompanied by bladder hyperactivity (urgency, frequency). Our results demonstrate peptidergic transmission under these conditions and provide a valuable tool to probe the mechanisms of plasticity at the cellular level.

POS-TUE-044

GENE THERAPY FOR THE PRESERVATION AND REGENERATION OF SPIRAL GANLION NEURONS (SGNS) AFTER DEAFNESS

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Introduction: The administration of exogenous neurotrophins to the deafened cochlea via mini-osmotic pumps can promote SGN survival and fibre regrowth. However, an additional consequence of pump-based neurotrophin delivery is that the peripheral fibres regrow in a disorganised manner and the survival promoting effects of neurotrophins is lost following the cessation of delivery, indicating that long-term neurotrophin treatment is required for sustained benefits. **Purpose**: We aim to provide localised, long-term sources of neurotrophins to the deafened cochlea that will not only promote long-term SGN survival but will also provide directional cues for fibre regrowth. **Methods**: We have administered a viral vector into the cochlea in order to initiate neurotrophin production by the transfected cells. Our initial studies have shown an increase in SGN survival and directed regrowth after 3 weeks of treatment, when introduced after 1 week of deafness, with the neurotrophin vector (n=4) compared to a control vector (n=4). Although these results are promising the longer-term effectiveness of this treatment remains to be established. Therefore, in our current study we are examining the effectiveness of neurotrophin gene therapy after long-term deafness and its long-term effects on SGNs. **Results**: Thus far we have shown that the introduction of neurotrophins via gene therapy after 4 (n=4) or 8 weeks (n=4) of deafness is unable to promote SGN survival in the guinea pig. **Conclusion**: This suggests that there is a level of structure and organisation required within the organ of Corti needed for neurotrophin gene therapy to be effective.

POS-TUE-045

ENHANCEMENT OF REGULATORY T CELLS FACILITATES RECOVERY FROM NEUROPATHIC PAIN IN RATS

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Purpose: T lymphocytes have been demonstrated to contribute to chronic neuropathic pain following peripheral nerve injury. CD4+/CD25+/FoxP3+ regulatory T (Treg) cells, are natural suppressors of T lymphocytes, and have been demonstrated to improve recovery in models of auto-immune disease. Here we assessed the effect of activating and expanding Treg cells on pain hypersensitivity and neuroinflammation following nerve injury. **Methods:** Rats underwent sciatic nerve chronic constriction injury (CCI) and were treated with superagonistic anti-CD28 antibody (CD28supA) to increase Treg numbers, or isotype control (0.5mg, I.V.) on day 0. We tested Treg numbers by flow cytometry, pain behaviours by sensory tests and immune cell activation in the injured nerve by immunohistochemistry (n=3-5/group). **Results:** Treatment with CD28supA resulted in a significant increase (1.6-1.8-fold) in Tregs in both spleen ($P<0.05$) and lymph nodes ($P<0.001$). Compared to sham, CCI reduced mechanical withdrawal threshold in both CD28supA and isotype groups between days 3 and 18, however on days 21 to 28 only the isotype group showed a significant ($P<0.001$) decrease in mechanical threshold. On day 28 the CD28supA group had a significant ($P<0.05$) increase in mechanical threshold compared to the isotype group. CD28supA treatment also led to a significant decrease in sciatic nerve T lymphocytes on day 28 ($P<0.05$), as well as a reduction in MHC class II cells on day 3 ($P<0.01$). **Conclusion:** Following CCI in rats, increasing Treg cell numbers leads to reduced allodynia and accelerated recovery from neuropathic injury, associated with a decrease in influx of inflammatory T lymphocytes and MHC class II cells. These data suggest that activation of Treg cells may be effective in the treatment of neuropathic pain.

POS-TUE-046

THE EFFECTS OF A TRANSIENT ELEVATION OF INTRAOCULAR PRESSURE UPON RETINAL FUNCTION AND STRUCTURE

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Purpose: The mechanisms of glaucomatous damage to retinal ganglion cells are still poorly understood. The purpose of this study was to assess the functional and histological effects of a sustained, but short-term, elevation of intraocular pressure in a transgenic mouse expressing cyan fluorescent protein in ganglion cells. This model allows *in vivo* longitudinal and *in vitro* analysis of ganglion cell numbers. **Methods:** A bolus of indocyanine green was injected i.v. into B6.Cg-Tg(Thy1-CFP)23Jrs/J mice (n=16). The episcleral veins adjacent to the limbus were photocoagulated with an 810nm diode laser to induce unilateral ocular hypertension. Of the 16 treated mice, 11 maintained an IOP of above 22mmHg for 20 days. The remaining 5 mice that displayed a transient elevation of IOP were analysed in the current study. Each mouse in this group displayed an individual IOP:time profile. Retinal function was assessed by scotopic electroretinography (ERG) and individual retinal cell types visualized by immunohistochemistry. Thy1-CFP-positive ganglion cells were observed by fluorescence microscopy. **Results:** During the period of transient IOP elevation, the ganglion cell-derived ERG scotopic threshold response was significantly affected. The positive component (pSTR) was suppressed by up to 80% (dependent upon IOP), compared with the non-treated contralateral eye. The pSTR partially recovered if the elevation of IOP was short (<5 days). However, the pSTR remained suppressed at day 35 when IOP was elevated for a period of 20 days. Similarly, there was no apparent loss of ganglion cells when the IOP was elevated for <5 days. A short, transient IOP elevation induced sustained GFAP expression in Müller cells but did not alter PKCα immunoreactivity in bipolar cells. **Conclusion:** These data suggest that IOP-mediated ganglion cell dysfunction precedes cell death and is potentially reversible if IOP is lowered promptly.

POS-TUE-047

MECHANO- AND CHEMOSENSITIVITY OF UNSPECIALISED AXONS OF PASSAGE IN COLONIC NERVES OF THE GUINEA PIG

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Purpose: Visceral mechanoreceptors have specialized endings at which they transduce mechanical stimuli. Intraganglionic laminar endings (IGLEs) are transduction sites of low threshold vagal mechanoreceptors. Varicose branching endings on intramural and extramural arteries transduce higher threshold mechano-nociceptive stimuli. Both can be activated by local distension, von Frey hairs and chemical mediators. Here we tested whether unspecialized axons-of-passage are comparably mechano- and chemosensitive. **Methods**: Extracellular recordings were made, in vitro, from the proximal end of unspecialized nerve trunks which traverse the colonic mesentery, uninterrupted, for 15–20mm. Biotinamide filling was then used to label recorded axons. **Results:** Recordings were made from 20 nerve trunks (n=10). Von Frey hair probing repeatedly evoked action potentials (median threshold; 10mN; 20 nerves, n=10). Distension also activated axons in nerve trunks, with thresholds of 40-50mN. The proportion of intact nerve trunks with axons sensitive to capsaicin: 17/20, to 5-HT: 5/20, to DMPP (nicotinic agonist): 6/20, to ATP: 6/20 and to KCl: 8/8. Biotinamide dye fills (37 nerve trunks, n=12) revealed just 2 axons which showed ramifying varicose axonal specializations in the mesentery proper. No specialized axonal structures were associated with blood vessels, fat or lymphatics. Dye fills consistently revealed axonal damage caused by stiff von Frey hairs: large blebs were visible in nerve trunks probed with von Frey hairs of 20mN or greater; often, axonal continuity was disrupted. **Conclusions:** Unspecialized, smooth axons-of-passage in colonic nerve trunks can be repeatedly activated by stiff von Frey hairs which also cause morphologically detectable damage. They are also chemosensitive and may be activated by high amplitude distension.

POS-TUE-048

RESPONSE ADAPTATION IN PRIMARY AND SECONDARY SOMATOSENSORY CORTICAL REGIONS IN THE CAT TO DUAL FREQUENCY VIBROTACTILE STIMULI APPLIED TO THE GLABROUS SKIN OF THE FOREPAW

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Purpose: The response adaptation in the cat's primary (SI) and secondary (SII) somatosensory regions of cortex to vibrotactile stimuli of high and low frequency was investigated using multichannel penetrating electrodes. **Methods:** Sinusoidal vibrotactile stimuli of 20Hz (or 23Hz), 200Hz or combined 20/200Hz (or 23/200Hz) of 4s duration were presented at various amplitude combinations to the glabrous skin of the forepaw of anaesthetized cats (n=3). Multi-unit spike activity was recorded from 64-channel NeuroNexus arrays inserted into contralateral SI and SII, in the region receiving input from the glabrous skin of the forepaw. **Results:** In SI, where a response was observed, an exponential decrease in the response representing stimulus adaptation could be identified. In SII, the response showed a slight increase for the first 150-200ms before exhibiting exponential adaptation. Response adaptation was most prominently for the first 2s of the stimulation. The decay profile remained largely the same across the electrode sites of the same cortical region with only minor alterations with respect to the dual frequency stimulus combination. **Conclusion:** Current models of the interactions between SI and SII suggest inhibitory feedback from SII to SI, which may be consistent with the observed difference in response profiles over the first 200ms.

POS-TUE-049

EFFECT OF A NOVEL PEPTIDE DISPLAYING NEUROPROTECTIVE ACTIVITY WITHIN THE HYPOXIC RETINA

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PURPOSE: To characterize a neuropeptide (US provisional 20100316) with brain-related neurogenic, neuro-migratory and neuronal differentiation properties in the intervention of hypoxia and ischaemia/reperfusion retina. **METHODS:** Retinal explants were incubated in a modified brain buffer mimicking hypoxic damage by bubbling it in 95%N2/5%CO2. The explants (n=4 per condition) were incubated in one of three solutions: modified brain buffer, 100fM neuropeptide or 100pM neuropeptide in brain buffer. After 40min the tissues were fixed and processed for immunocytochemistry. To assess the *in vivo* effect of the neuropeptide, animals were anaesthetized and an ischemic insult was induced (n=5) by elevating the rat intraocular pressure to 120mm Hg for 1.5 hours. Reperfusion occurred for 12hs in the dark, followed by recording retinal activity using an electroretinogram (ERG). Left eyes were injected with 2 picomoles of neuropeptide. ERG recordings were repeated and subsequently the eyes were removed and processed for immunocytochemistry. Rabbit anti-GFAP was employed to detect retinal stress and TUNEL cell detection kit was employed to detect cell death. **RESULTS:** *In vitro* application of the peptide to hypoxic retinal explants protected the tissue against cell death and reduced significantly the extent of GFAP labeling while in the hypoxic retina there was increased expression of GFAP and neuronal cell death. There was a dose-response effect of the neuropeptide showing that 100fM of the neuropeptidewas more effective at reducing the extent of GFAP expression compared with 100 pM of peptide. Analysis of the electrical activity of the retina showed that IOP followed by 12 hrs of recovery significantly reduced ($p<0.05$, ANOVA) the retinal activity. In the presence of 100fM neuropeptide the retinal activity was restored close to normal values compared with a time matched ischaemic control retina. **CONCLUSION:** The novel neuropeptide represents a candidate neuroprotective molecule in the injured hypoxic retina.

POS-TUE-050

ELLIPTICAL POLARIZATION VISION IN A STOMATOPOD CRUSTACEAN

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The ability to discriminate circularly polarized light, namely circular polarization vision, has been found in several species of stomatopod crustaceans. This visual system achieves this using a structure that acts as a wave retarder, which converting circularly polarized light of opposite handedness into linearly polarized light of perpendicular e-vector angles. Retardance is dependent on the length of these structures, and due to the limit of available space, a structure that can create quarter wave retardation may be too large to fit in the eyes of some smaller stomatopod species. **Purpose:**Here we investigate the circular polarization vision in one species of stomatopod, *Haptosquilla trispinosa*, which possess relatively small eyes (<2mm in diameter). **Methods:**Using intracellular electrophysiological recording to measure polarization sensitivities from retinular cells, **Results:**we find that any photoreceptor cell that is sensitive to circularly polarized light will also discriminate the e-vector angle of linearly polarized light. Intracellular dye injection during physiological recording reveals that these photoreceptors are retinular cells 1-7 (R1-R7) in the mid-band rows 5 and 6, identical to those circularly polarization sensitive cells in other species. From previous studies, we have identified the rhabdomere of the 8th retinular cell (R8) as the optical structure which converts circularly polarized light into linear. In the eyes of *H. trispinosa*, the space available is so limited that allocation of extra room for R8 (i.e. a better retarder) means losing ground for R1-R7 (i.e. reducing quantum catch). **Conclusion:**Keeping some circular polarization vision, *H. trispinosa* have evolved a compromise solution, a visual system that is best tuned to discriminate elliptically polarized light.

POS-TUE-051

DISTRACTIBILITY AND COLLICULAR RESPONSIVITY IN THE GENETICALLY HYPERTENSIVE RAT

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Purpose: Attention deficit hyperactivity disorder (ADHD) is characterised by increased distractibility. Accumulating evidence suggests that this core symptom may be caused by a dysfunction of the superior colliculus (SC) – a structure intimately linked with distractibility. We hypothesized that an animal model of ADHD would show increased distractibility on tasks related to SC function and that the SC would be hyper-responsive to sensory inputs. **Methods:** We investigated the response of genetically hypertensive (GH) and Wistar (WI) rats (n=16/strain) to a distracter in an open field and a hidden platform water maze. We also measured neuronal activity to light flash stimuli in the superficial layers of the SC. **Results:** GH rats spent more time near the distracter in the open field and less time near the distracter in the water maze than WI rats. GH rats also showed greater SC activity in response to visual stimuli across a range of stimulus intensities, and d-amphetamine reduced visual responses less markedly in GH rats. **Conclusion:** These findings indicate that GH rats are more distractible on tasks related to collicular functioning and that they may have an underactive anterior attentional system. Increased distractibility at the behavioural level may reflect the enhanced SC visual responsiveness seen in GH rats. Although GH rats were less sensitive to d-amphetamine than WI rats, d-amphetamine lowered visual responsiveness in GH to the baseline activity level of controls, suggesting that the SC may be a therapeutic target for the treatment of ADHD.

POS-TUE-052

COLOUR VISION AND RETINAL TOPOGRAPHY IN A POLLINATING BIRD, THE RED WATTLEBIRD ANTHOCHAERA CARUNCULATA

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Purpose: Birds that rely on nectar as a main source of energy need to discriminate different colour signals in flowers and, while feeding, to be vigilant for predators. Vision is therefore an important sensory modality for nectarivorous birds. Honeyeaters (Maliphagidae) represent a group of nectar-feeding passerines that are important for pollination but little is known of their visual capabilities. **Methods:** Retinal whole mounts were used to determine the distribution of ganglion cells, and microspectrophotometry to determine the spectral characteristics of photoreceptors and oil droplets in the retina of the red wattlebird, *Anthochaera carunculata*. **Results:** The topography of neurons in the retinal ganglion cell layer revealed a temporal area and a central fovea that may be advantageous to increase binocular vision for the perception of flowers and for the detection of objects approaching the lateral visual field. Microspectrophotometry of photoreceptor outer segments revealed a rod with peak wavelength at 506 nm. Spectrally distinct classes of cones contained visual pigments maximally sensitive to long (LWS: 559 nm for single cones, 562 nm for double cones), middle (MWS: 506 nm) and short (SWS:459 nm, VS: 406 nm) wavelengths. Each subtype of single cone was associated with a different oil droplet with cut-off at 570 nm (LWS), 515 nm (MWS), 420 nm (SWS) and 311 nm (VS), but only the principal member of double cones possessed an oil droplet (cut-off at 483 nm). **Conclusion:** The retina of this species shows the basic complement of photoreceptors conserved amongst passerines. It differs however by the presence of a violet (VS) rather than an ultraviolet sensitive pigment.

POS-TUE-053

THE EFFECT OF OXYGEN-GLUCOSE DEPRIVATION ON SPONTANEOUS ACTIVITY IN THE DEVELOPING COCHLEA

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Prior to the onset of hearing, the developing auditory system undergoes spontaneous electrical activity to retain and refine neural connections. This is important for developing and maintaining auditory circuits in the absence of sound. However, the exact processes behind this are poorly understood. The developing mammalian cochlea contains a group of epithelial cells known as the Kölliker's organ. These cells periodically release ATP, depolarising inner hair cells and its supporting structures by interactions with purinergic receptors. Research has shown a correlation between these electrical events, intracellular Ca^{2+} concentration, and optical changes within the Kölliker's organ. **Purpose:** This study explored the effects of oxygen-glucose deprivation (OGD) on optical changes regarded as an index of spontaneous activity. **Methods:** Organ of Corti from the apical turn of developing Wistar rat cochlea (P7-P13) was studied using real-time imaging. OGD was induced by altering the composition (glucose to sucrose) and gas exposure (oxygen to nitrogen) of the bathing fluid. Spontaneous optical events were analysed as average changes in pixel intensity during control, OGD, and reperfusion periods. **Results:** An overall decrease in optical change (frequency and amplitude combined) was observed between control (n=5) and OGD treated (n=6) animal tissue (P<0.05). Within each of the OGD-treated cochlear turns, a significant increase in optical changes was present following reperfusion (P<0.05), indicating the return of spontaneous activity in the Kölliker's organ. **Conclusion:** The results indicate that the spontaneous activity in Kölliker's organ is vulnerable to energy deprivation, possibly indicating a conservation of intracellular ATP in the presence of metabolic stressors such as OGD. This research was approved by the University of Auckland Animal Ethics Committee.

POS-TUE-054

RESPONSE OF NEURONS IN AREA MT OF MARMOSET TO MOVING TEXTURES AND NATURAL IMAGES

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Purpose: The tuning properties of motion-selective neurons in area MT of primate visual cortex are traditionally measured using simplified stimuli such as gratings. It is not clear how these neurons respond during motion that more closely approximates that in normal viewing, where images have a broad range of orientations. **Methods:** Extracellular single-unit recordings were made from neurons in the middle-temporal area of anaesthetized (sufentanyl forte, 9 µg/kg/hr) male marmosets (n = 5). The stimulus was a photo of a natural scene, or textures whose orientation content was parametrically varied. In each case responses were obtained for each of 12 directions. Where possible neurons were classified as 'pattern cells' or 'component cells' using standard techniques. **Results:** 21 pattern cells, 17 component cells and 23 unclassifiable cells responded over 10 impulses/s and were used for analysis. For component cells, response amplitude was largely independent of the image used to stimulate the receptive field, and bandwidth increased as more orientations were introduced into the synthetic textures, or the natural images. For pattern cells, response amplitude increased with the range of orientations present in the image, and bandwidth was independent of the image used. Unclassifiable cells showed intermediate response patterns. **Conclusion:** In component cells each orientation acts as if it introduces a new direction into the stimulus. The motion selectivity of pattern cells did not depend on the type of image used to measure it. This suggests that pattern cells provide a stable representation of direction during natural stimulation.

POS-TUE-055

SPECIALISED SENSORY INNERVATION OF THE CLITORIS AND EXTERNAL GENITALIA OF FEMALE GUINEA-PIGS

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Purpose. We used multiple-labelling immunohistochemistry and confocal microscopy to characterise the sensory innervation of the external genitalia and surrounding structures of female guinea-pigs (n=8). **Methods**. Large diameter sensory fibres were labelled with immunoreactivity to neuron-specific enolase (NSE) or vesicular glutamate transporter 1 (VGluT1). Peptidergic sensory fibres were labelled with immunoreactivity to calcitonin gene-related peptide (CGRP) and substance P. C-Fos immunoreactivity was used to identify activated neurons in the spinal cord of anaesthetised guinea-pigs after stimulation of the genitalia. **Results.** Large complex NSE-immunoreactive nerve endings were abundant in dermal papillae of the clitoris. Each ending was accompanied by fine fibres immunoreactive for CGRP but neither substance P nor VGluT1. More simple NSE-immunoreactive endings occurred within dermal papillae of the labia and anal canal. Large endings were rare in pudendal or perineal hairy skin. Fine intra-epithelial fibres immunoreactive for NSE but not CGRP were abundant in hairy skin but rare in the clitoris. No large NSE-immunoreactive endings occurred in the cervix but fine varicose fibres immunoreactive for both CGRP and substance P were common. Mechanical stimulation of the genital tract activated c-Fos expression mainly in lamina 2 of sacral spinal cord. **Conclusions.** These observations illustrate the highly specialised nature of sensory fibres in the clitoris compared with surrounding tissues. Fine fibres containing CGRP but not substance P within each mechanoreceptor complex probably contribute to unique genital pain sensation.

POS-TUE-056

PRIOR ABLATION OF IB4 BINDING NOCICEPTORS SIGNIFICANTLY REDUCES MICROGLIAL ACTIVATION IN THE RAT DORSAL HORN FOLLOWING CHRONIC CONSTRICTION INJURY OF THE SCIATIC NERVE

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Purpose: A population of nociceptors express the proteoglycan versican and bind IB4. It has recently been revealed that $IB4^+$ nociceptors are involved in the establishment of mechanical allodynia through a peripheral MCP-1 dependent mechanism. However, it remains uncertain whether $IB4^+$ nociceptors exert similar influence in the spinal cord. Studies have shown that activated microglia play an important role in the development and maintenance of neuropathic pain. Additionally, the biochemical profile of $IB4^+$ nociceptors suggests that they may contribute to the microglia-nociceptor interface in the dorsal horn. We set out to determine whether post-CCI expression of CD68 (ED1), a marker of activated microglia, was altered in $IB4^+$ nociceptor-deficient spinal circuits. **Methods:** Rats were divided into three groups (n=4/group): one group was surgically naive, another underwent CCI only, while the last was injected with 3µl of the $IB4^+$ nociceptor selective neurotoxin IB4-SAP (600µg/ml) 21 days prior to CCI (SAP+CCI). At 7 days post-CCI rats were perfused, spinal cords removed and processed to visualize dorsal horn IB4 binding and ED1 immunoreactivity. **Results:** SAP+CCI rats exhibited an almost complete depletion of dorsal horn IB4 binding. Moreover, SAP+CCI rats were found to have a significantly lower increase in CD68 than CCI only rats (83.1% versus 161.8%), relative to the uninjured side. No significant difference was found in naive rats (1.8%). **Conclusion:** These results suggest that the influence of $IB4^+$ nociceptors extends outside their immediate neural milieu and that they contribute to a pathway able to effect long term changes to the microglial profile of the dorsal horn of the spinal cord.

POS-TUE-057

HISTAMINE IN THE DEVELOPMENT AND MAINTENANCE OF THE RETINA

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Purpose: Histamine is known to be involved in the local immune response and in neurotransmission in the brain. Histamine and its receptors have been found in the mammalian retina but little is known about their function. We have analysed mouse mutants deleted for the gene for histidine decarboxylase (HDC-KO), the enzyme which catalyses the final step in the production of histamine. These mutants specifically lack histamine. We made the surprising observation that the HDC-KO mice show an aberrant phenotype in the outer retina, reminiscent of photoreceptor diseases. Our aim was to characterize the nature and time course of this phenotype in detail. **Methods**: Wild-type, (n=15) and HDC-KO (n=15) mice from postnatal day 3 (P3) to 14 weeks were used. Retinae were sectioned (paraffin, cryostat) and immunostained or stained as wholemounts. Using antibodies against specific cell types and structures, changes in photoreceptors and inner retinal neurons were examined. Retinal function was measured using the electroretinogram using a paired-flash paradigm to separate rod and cone responses. **Results**: 1. immunohistochemistry revealed aberrations (whorls, rosettes) in the photoreceptor layer of the HDC-KO retina, mainly in the ventro-temporal retina. 2. The aberrations occurred as early as P4. 3. The outer limiting membrane, which is an adhesion belt formed by photoreceptors and Mueller cells, was disrupted in the HDC-KO retina. 4. Inner retinal function was altered in the HDC-KO mice, but photoreceptor responses were normal. **Conclusions:** The majority of human diseases resulting in blindness affect photoreceptors. If we can understand the factors (such as histamine), which regulate the development of photoreceptors, it may be possible to use this knowledge to develop effective treatments for regeneration of photoreceptors in blindness.

POS-TUE-058

CHARACTERISATION OF CONNEXIN 43 IN A LIGHT EXPOSED ANIMAL MODEL OF AGE-RELATED MACULAR DEGENERATION

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PURPOSE: To investigate the role of connexin 43 (Cx43) in light mediated retinal degeneration as a model for age related macular degeneration. METHODS: Adult Sprague-Dawley rats (males and females) were used for this study. Both control (n=6) and experimental group (n=6) were raised in normal light conditions (300 lux) in a 12 hours day/night cycle. The experimental group was exposed to intense light (2700 lux) for 24 hours. Immediately post-exposure, the animals were anaesthetized and the eyes were dissected out and processed for immunofluorescence labeling. Antibodies against Cx43 and markers associated with oxidative stress (nitrotyrosine), inflammation (CD45) and TUNEL cell death kit were used to investigate damaging/survival mechanisms in the retina, choroid and sclera. Intensity of labeled area was measured using Image J in tissues processed and observed under identical conditions. RESULTS: There was increased expression of Cx43 in the light exposed retina, mainly in the nerve fiber layer and a subpopulation of amacrine cells when compared to control retina. In the retinal pigmented epithelium (RPE); there was significant up-regulation of Cx43 with altered morphology of these cells. Cx43 expression colocalised with both nitrotyrosine and CD45 expressing cells in the choroid and sclera. TUNEL labeling was observed in the photoreceptor layer of the light damaged retina. CONCLUSIONS: The light damaged retina presents with evidence of inflammation and oxidative stress concomitantly with the onset of photoreceptor death. The earliest events in the process of retinal degeneration coincide with increased Cx43 expression. There is also a major contribution of cell-cell communication in RPE pathology that remains to be investigated.

POS-TUE-059

EXPRESSION ANALYSIS OF SPHINGOSINE 1-PHOSPHATE SYNTHESISING ENZYMES AND RECEPTORS IN RESPONSE TO INFLAMMATION AND NERVE DAMAGE

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Purpose & Methods: To investigate how the mRNAs for Sphingosine kinase1 and 2 (Sphk1, 2) and sphingosine 1-phosphate (S1P) receptors, S1P1-3, are regulated under conditions of inflammatory pain (Complete Freund's Adjuvant (CFA) hindpaw injection) and nerve damage (sciatic nerve transection, ScNT). RT-qPCR was performed using total RNA from DRG L3-L5 ipsi- and contralateral to the site of inflammation (days 1, 2) or damage (days 2, 4, 6). Indicators for inflammation were: swelling of the ipsilateral paw and increased NPY mRNA levels. Successful ScNT was demonstrated by increased ipsilateral NPY mRNA expression. Immunohistochemical analysis after ScNT showed a strong nuclear labelling for the transcription factor ATF-3 in ipsilateral DRG. Results: ScNT induced a significantly lower expression of S1P1 and S1P3, after 2 and 6 days, of Sphk1 and Sphk2 after 6 days but a higher expression of S1P2 after 4 days in ipsilateral compared to contralateral DRG. NPY mRNA was highly upregulated in all animals (n = 5-8/day, up to 500fold). Injection of CFA but not saline increased swelling of the ipsilateral paw in all animals whereas NPY mRNA expression levels surprisingly did not increase in all ipsilateral DRG. The mRNA for Sphk1 was significantly reduced in DRG that showed increased NPY mRNA levels (d1, n = 5, d2 n = 6), whereas the mRNAs for Sphk2 and S1P1-3 remained unchanged. Conclusion: Nerve damage changes the expression of S1P synthesising enzymes and receptors, whereas inflammation in particular targets the Sphk1 enzyme.

POS-TUE-060

RETINAL DYSFUNCTION IN AN ANIMAL MODEL OF RETINOPATHY OF PREMATURITY

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Purpose: Retinopathy of prematurity (ROP) is characterised by retinal neovascularisation and visual impairments following high oxygen treatment of premature infants. Changes in retinal glia and neurons have been reported in oxygen-induced retinopathy (OIR), an animal model of ROP. This study examines whether the angiotensin II type-1 receptor inhibitor, valsartan, prevents these changes. **Methods**: Newborn Sprague-Dawley rats were either exposed to 80% oxygen until postnatal day 11 (P11) and then room air until P18 (OIR; n=) or room air from P0-18 (control). Control and OIR rats either received no treatment or valsartan (4,10 or 40mg/kg/day intraperitoneal) from P12-P17 (n=10-12). Retinal function was assessed with twin-flash electroretinography (ERG) at P18. IbA1 (microglia) and IB4 (vasculature) immunohistochemical labeling was used and micrographs taken using a Zeiss LSM 5 confocal microscope. **Results**: OIR animals presented with prominent neovascular tufts extending into the vitreous while the peripheral retina remained avascular. Valsartan attenuated the neovascularisation however did not encourage normal angiogenesis. Neuronal function was significantly reduced in OIR. The rod photoreceptor response (a-wave) and rod post-receptoral response (b-wave) were significantly reduced in OIR rats compared with controls. The oscillatory potentials were also reduced. Valsartan did not prevent neuronal dysfunction. Retinal microglia were significantly activated and increased in number across the whole retina in OIR. Following valsartan treatment, numbers and morphology resumed to that of the control at P18. **Conclusion**: Our results indicate that the AT1-R antagonist valsartan prevents pathological angiogenesis and microglia activation however does not improve neuronal dysfunction. Further work is required to understand the underlying mechanisms causing neuronal dysfunction during ROP.

POS-TUE-061

N-TERMINAL RESIDUES OF THE CALCITONIN RECEPTOR INVOLVED WITH AMYLIN INTERACTIONS WITH THE AMYLIN 1 RECEPTOR

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Purpose. Amylin is a 37 amino acid peptide which is co-released with insulin from the pancreas to act on the circumventricular organs. Amylin is involved in glucose homeostasis and the control of body weight. An amylin analogue is currently used to treat diabetes and amylin/leptin co-therapy is showing promise for the treatment of obesity. Nevertheless, non-peptide agonists of amylin would be potentially better therapeutic prospects. To develop these, we need to understand how amylin interacts with its receptors. The Amylin 1 (AMY1) receptor is a heterodimer of the calcitonin receptor (CTR), a G protein-coupled receptor (GPCR) and receptor activity-modifying protein 1 (RAMP1). It is thought the extracellular N-terminal domains of both receptor components contribute to high affinity amylin binding. While residues in the RAMP1 N-terminus have been identified that contribute to ligand binding at this receptor, few individual residues have been identified within CTR. **Methods.** As truncation of the first 47 amino acids of CTR does not reduce amylin potency at the AMY1 receptor we have investigated the role of individual residues immediately beyond this region by alanine scanning mutagenesis of CTR. These mutants have been expressed with RAMP1 in Cos7 cells and the function of the mutant AMY1 receptors determined by measurement of cAMP production in response to amylin and related peptides. The cell surface expression of receptors was determined by ELISA. **Results.** The mutation Y53A was found to significantly reduce peptide potency (n=3), compared to the wild-type receptor, whilst not altering cell surface expression. **Conclusion.** This suggests that Y53 of CTR plays an important role in peptide interactions with the AMY1 receptor.

POS-TUE-062

A MODEL FOR NEURONAL PROPERTIES IN PRIMARY VISUAL CORTEX

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Purpose. Our aim was to describe the simplest possible model capable of reproducing three fundamental properties – direction selectivity, orientation selectivity, and complex responses – of neurons in primary visual cortex. Further, we aimed to match the model's population properties with those found empirically in a cortical column. Methods. The model consists of two sub-cortical channels (one on- and the other off-centre) and four sub-cortical stages: photoreceptors, bipolar cells, X-type ganglion cells, and lateral geniculate relay cells. Primary visual cortex is modeled with three stages, each comprising a spatial grid of neurons. Activity converges from the geniculate to the first stage, and from each cortical stage to the next, with a Gaussian spatial spread. All neurons in the model are simulated as linear low-pass temporal filters, with one differential equation per neuron; the output of cortical neurons is rectified. Model parameters are taken from published studies of the cat's visual system. Results. First, cortical neurons in the model are direction selective because of small differences in the signal processing time in the two sub-cortical pathways. A frequency histogram of the direction selectivity index for neurons in the first cortical stage has a similar form to that obtained in the laboratory. Second, cells in the second and third cortical stages are complex-like due to rectification. Frequency histograms of the modulation/mean ratio reproduce the elements of empirical measurements. Third, cortical receptive fields in the model had on- and off-subfields, endowing them with orientation selectivity. However, extra pairs of sub-cortical channels had to be added to reproduce the narrow orientation tuning curves seen in the laboratory. Conclusion. The model simulates both the existence and diversity of three fundamental properties of primary visual cortex.

POS-TUE-063

CIRCULAR POLARISATION SENSITIVITY IN STOMATOPOD CRUSTACEANS: PREDICTING THE DIMENSIONS OF R8 QUARTER-WAVE RETARDERS

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The visual system of stomatopod crustaceans (mantis shrimps) demonstrates some of the most sophisticated specialisations of compound eye structures found in nature. Aside from an advanced colour vision system containing more photopigment types than any other known in nature, these species have been shown to possess the ability to discriminate both linearly and circularly polarised light. The circular polarisation detection system depends on the use of optical retardance structures in the eye which convert circular to linear polarised light. Previous studies have demonstrated that the structures responsible for this are the rhabdomeres of the 8th retinular cells (R8) located in midband rows 5 and 6. These R8 cells contain sub-wavelength scale microvillar membranes whose birefringent properties produce the optical retardance effect. In this study we used mathematical models to predict the optimal dimensions of R8 rhabdomeres for converting circular to linear polarised light. We compared these predictions to actual R8 dimensions measured for multiple species of stomatopod (n=8), and link these findings to elecrophysiological recordings. We found that the R8 rhabdomeres of some species had dimensions that were consistent with full quarter-wave retarders. However, other species, particularly those with small eyes, had shorter R8 rhabdomeres corresponding to eighth-wave retarders, which would only be able to convert circular to elliptical polarised light, a compromise solution for space-limited eyes.

POS-TUE-064

RECEPTIVE FIELD PROPERTIES OF MOUSE RETINAL GANGLION CELLS ARE AFFECTED BY INHIBITION FROM INNER PLEXIFORM LAYER

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Retinal ganglion cells (RGCs) receive excitatory inputs from bipolar cells and inhibitory inputs from amacrine cells, which are critical for determining their spatial and temporal properties. **Purpose**: To investigate the role of direct inhibitory input and presynaptic inhibition on the spatial-tuning properties of RGCs and the role of directionally tuned excitatory and inhibitory inputs on generating direction selectivity in RGCs. **Methods**: A hybrid RGC-computer circuit was implemented via dynamic-clamp, to assess the impact of excitatory and inhibitory inputs onto RGCs in whole-mount retinae. Light-evoked synaptic conductances recorded in response to spots of different sizes in control conditions and under TTX were injected into RGCs. RGC responses were also assessed using digitally synthesised directionally tuned conductances, modelled on physiological responses of direction selective RGCs. **Results**: Recordings were obtained from A, B and C RGC subtypes (n=34). The size-tuning curves showed peak responses for small spots (150µm), which decayed for larger spots (1400µm). Independent and combined blockade of direct inhibitory input and presynaptic inhibition by TTX relieved surround inhibition. Injection of directionally tuned conductances showed that direct inhibitory inputs modulate response strength in the preferred direction but are critical for conferring direction selectivity in the null direction, as they obliterate otherwise occurring responses. **Conclusion**: Our findings indicate that inhibition generated in the inner plexiform layer by spiking amacrine cells increases the sharpness of spatial-tuning curves of ganglion cells. We also found that in direction-selective ganglion cells, direct inhibition is crucial for preventing spike generation for stimuli moving in the null direction.

POS-TUE-065

OCELLAR INPUTS TO MOTION-SENSITIVE DESCENDING NEURONS IN THE HONEYBEE, APIS MELLIFERA

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Honeybees, like most flying insects, have three ocelli (simple eyes) located on the top of the head, in addition to two large compound eyes. **Purpose:** Many experiments have been conducted to reveal the function of the ocelli in the visual system. However, the exact function, and the information computation between ocelli and compound eyes are still unclear. **Methods:** In this study, I investigate the morphological and optical characteristics of honeybee ocelli using semi-thin sections and focal length measurements on both median and lateral ocelli, along with scanning electron microscopy. Intracellular electrophysiology experiments were also carried out on honeybee ocellar descending neurons and motion-sensitive descending neurons to understand the information processing between ocelli and compound eyes. Cell responses to different stimuli were recorded, with and without ocelli covered. **Results:** For the identified motion-sensitive descending neuron DNII2, responses to flash stimuli were delayed when the ocelli were covered. With the ocelli uncovered, responses to motion stimuli were characterized by an excitatory rebound for the anti-preferred direction. **Conclusion:** These results suggest that there are ocellar inputs to the motion-sensitive descending neurons.

POS-TUE-066

FUNCTIONAL CONNECTIVITY IN AUDITORY CORTEX

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Purpose: Primary auditory cortex (AI) is known to undergo plastic reorganisation in response to changes in peripheral input. It is generally accepted that these changes depend in part on long-range horizontal projections within AI. We sought to investigate functional connectivity in AI by studying outward travelling local field potentials (LFPs), as recently described in visual cortex[1]. **Methods**: Single- (SU) and multi-unit (MU) spike activity and LFPs and were recorded from multi-channel electrode arrays implanted in AI in three normal-hearing adult cats. Spike-triggered averages of LFPs were generated using the spike activity from each recording site as the trigger (i.e., "reference electrode"; MUs = 96, SUs = 26). For each averaged LFP, the latency to peak and the response amplitude were measured. On the basis of these data, outward travelling LFP waves were characterised by increasing latency, and decreasing amplitude, with distance from the reference electrode. **Results**: Travelling LFP waves were observed radiating from 17% of MU and 19% of SU recording sites. The average propagation velocity was 1.1 ± 0.2 ms-1 (mean ± standard error of the mean) for MUs and 0.7 ± 0.2 ms-1 for SUs. The length constant of the travelling LFPs was 2.4 ± 0.8 mm for MUs and 3.1 ± 0.8 mm for SUs. There was no significant difference in either metric between LFPs referenced to MUs or SUs (paired T-test; n = 4; P's > 0.12). **Conclusion**: Measurement of outward travelling LFP waves will provide a valuable tool in assessing functional connectivity in AI. **References**: [1] Nauhaus et al. 2010 Nature Neuroscience **12**:70-76.

POS-TUE-067

ANIMAL MODELS OF INFLAMMATORY BONE PAIN

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The aim of the present study was to develop animal models of acute and persistent inflammatory bone pain and determine if they can be used to test the efficacy of pharmacological therapeutics. Sprague-Dawley rats (n=69) were anaesthetized with halothane and inflammation was induced by injection of either Complete Freund's Adjuvant (CFA) or carageenan into the tibial medullary cavity. The percentage of weight bearing on the injected hindlimb was determined using an incapacitance meter and a decrease in percentage weight bearing was taken as an index of pain. CFA injection resulted in a persistent pain profile that consisted of a decrease in ipsilateral weight bearing for up to 7-10 days post-injection, and a slow return to normal over the next 2 weeks. Carageenan injection resulted in an acute pain profile, with a decrease in weight bearing at 15 minutes and at 5 hours, and then a return to normal by day 2. The percentage of ipsilateral hindlimb weight bearing at the peaks of CFA induced pain (day 7) and carageenan induced pain (15 min and 5 hours) was significantly lower than both the pre-injection values for the same animals ($P<0.05$) and the values for the saline injected and sham (bone exposed but no injection) control groups at the same time-points ($P<0.05$). Peak CFA induced pain was abrogated by the COX II inhibitor carprofen (5 mg/kg s.c.). These data indicate our models provide reliable and robust measures of acute and persistent inflammatory bone pain, and can be used to test the efficacy of pharmacological treatment for inflammatory bone pain.

POS-TUE-068

CHARACTERISATION OF PAIN BEHAVIOUR, SPINAL NEUROCHEMISTRY AND GLIAL POPULATIONS IN A MOUSE ANTIGEN-INDUCED ARTHRITIS MODEL

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Studies of arthritis in mice typically focus on either joint disease or joint pain. Surprisingly, each field employs different models. Antigen-based models are preferred for studying joint disease and show greater relevance to human arthritis, whereas adjuvant-based models are preferred in pain studies. **Purpose:** To establish the antigen-based methylated bovine serum albumin interleukin 1 (mBSA/IL1) mouse model of arthritis and study how this model affects i) pain related behaviour; ii) sensory neuropeptides; and iii) microglia and astrocytes in the spinal cord. **Methods:** Arthritis was induced in anesthetized (isoflurane 2%) animals (n=4) as follows: Day 1 intra-articular injection of mBSA (right knee) and subcutaneous IL-1 injection (right hindpaw); Day 2 subcutaneous IL-1 injection (right hindpaw); Day 3 subcutaneous IL-1 injection (right hindpaw). Sham animals (n=4) received a vehicle only intra-articular injection and the same IL-1 regimen. Pre arthritis values for knee diameter, knee sensitivity, gait, thermal and mechanical paw withdraw thresholds were compared with post arthritis values (Day 7). Finally, spinal cords were removed (ketamine anesthesia, 100 mg/kg) and processed for substance P, CGRP, IBA1 and GFAP immunolabelling. **Results:** mBSA/IL1 arthritis significantly increased knee diameter (3.92 ± 0.05 mm vs 3.72 ± 0.03 mm, arthritis vs sham). Despite this, no significant differences were detected in knee or hindpaw sensory thresholds between groups. Gait analysis showed hindpaw stance was reduced in arthritic animals (1.91 ± 0.10 cm vs 2.31 ± 0.07 cm). None of the immunohistochemical markers were significantly different in arthritis versus sham tissue, however, there was a trend towards increased substance P, IBA1 (microglia) and GFAP (astrocyte) staining in arthritic spinal cords (n= 2, and 2). **Conclusions:** These preliminary results indicate that mBSA/IL1 arthritis may be useful for future studies of arthritic pain in a model with established relevance to rheumatoid arthritis.

POS-TUE-069

ON AND OFF RETINAL GANGLION CELLS (RGCS): MODELLING SYNAPTIC INPUT AND RESPONSE TO SINUSOIDAL STIMULATION

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Introduction: In degenerate retina (RD) rhythmic synaptic input drives ongoing oscillatory firing in ON and OFF RGCs at a frequency of 10Hz in the absence of sensory input. Maintained rhythmic activity is not synchronized between RGCs and is a characteristic feature of RD RGCs that is not seen in normal retina. ON and OFF RD RGCs also have different balance of inhibitory and excitatory inputs. **Aim:** The aim of this work is to model maintained synaptic input into ON/OFF RD RGCs, which extends our previous model of ON/OFF cells that was based on their intrinsic electrophysiology. The models are then used to examine responses of ON/OFF RGCs to sinusoidal stimulation currents. **Methods:** Numerical simulations of single-compartment Hodgkin-Huxley type neurons were carried out in NEURON. Synaptic input was modelled based on the experimental data in (Margolis and Detwiler 2008), the dynamics of excitatory and inhibitory currents were fit using polynomial curve fitting. The frequency-amplitude space of sinusoidal currents was systematically explored with stimulations of ON and OFF RGCs, the mean spike rate for ON/OFF cells was plotted as function of frequency and amplitude of sinusoidal stimulation. **Results:** Simulations showed that without stimulation both ON and OFF cells have continuous rhythmic bursts of spikes with an interburst beating frequency of 10Hz. The average firing rate of ON and OFF cells was 30Hz, which corresponds well to the experimental data of the overall mean rate of 24±3Hz. The model predicted that with sinusoidal stimulation OFF cells fire preferential over ON cells in a frequency band around 10Hz.

POS-TUE-070

GDNF FAMILY RECEPTOR ISOFORMS RET9 AND RET51 ARE DIFFERENTIALLY EXPRESSED IN THE OLFACTORY SYSTEM

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Purpose: The RET tyrosine kinase receptor is activated by glial cell line-derived neurotrophic factor (GDNF) family ligands, promoting neuronal survival and function. Previously, we reported the cellular localisation of the ligands, GDNF and neurturin, and RET in the olfactory system (Maroldt et al., 2005). In the current study, we examined expression of the two main protein isoforms of RET, RET9 and RET51, in the rat olfactory system to obtain evidence relating to their functional roles. **Methods:** Immunohistochemistry was employed to determine expression of the isoforms in olfactory tissue of adult and neonatal rats (n≥4 animals for all experiments). **Results:** RET9 was expressed by olfactory receptor neurones (ORNs) throughout the neuroepithelial sheet whereas RET51 was restricted to a subset of RET9-expressing ORNs in the ventromedial and ventrolateral regions. In the olfactory bulb, RET9 was primarily expressed by cell bodies and RET51 by a subset of these RET9-expressing cells. High levels of RET51 expression were also observed on axons in the olfactory nerve layer and presynaptic glomerular neuropil, but only in the ventromedial and ventrolateral regions of the bulb, which correlated with the predicted axonal projection from the ON. Furthermore, RET51 was expressed by dendrites in the external plexiform layer and the postsynaptic glomerular neuropil throughout the bulb proper independently of its presynaptic expression pattern. **Conclusion:** Our results suggest RET9 may be the predominant functional isoform in the ON while RET51 may play a more selective role in a restricted neuroepithelial region. In the bulb, RET9 is likely the main functional isoform while RET51 may be important in axonal and dendritic function/targeting and also might be more restricted in its zone of influence.

POS-TUE-071

RECOVERY OF NOCICEPTIVE THRESHOLDS FOLLOWING TARGETED ABLATION WITH IB4-SAP CO-INCIDES WITH INCREASED MICROGLIAL ACTIVATION IN THE RAT SPINAL CORD

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Purpose: Intrasciatic injection of IB4 conjugated to saporin (IB4-SAP) selectively ablates IB4+ nociceptors, resulting in an increase in nociceptive threshold by 7d. However, this increase appears to be transient with nociceptive thresholds returning to baseline by 21d despite persisting IB4+ nociceptor loss. Microglial activation after nerve injury contributes to neuropathic pain symptomology and as IB4-SAP treatment constitutes partial nerve injury in itself, it was of interest to determine whether IB4+ nociceptor ablation triggers microglial activation. **Methods:** All rats (n=28) were subjected to sensory testing before treatment, and every 3d thereafter. Experimental rats (n=20;4/group) were anaesthetised and the left sciatic nerve injected with IB4-SAP (3µl at 600µg/ml). Control rats included naives (n=4) and rats injected with un-conjugated IB4 (n=4). Injected rats survived for 7,14,21,28 or 35d and were assessed for sensory changes throughout survival and immediately before perfusion. Lumbar spinal cords were removed and processed for CD68 immunoreactivity (IR) to visualise activated microglia after various post-injection survival periods. **Results:** Analysis of IB4-SAP-induced sensory changes revealed an increase in nociceptive thresholds by 7d post-injection, followed by a sharp decrease in nociceptive thresholds between 12 and 18d (maximal at 14d). At 21d post-injection nociceptive thresholds were again increased, nearing baseline levels by 28 and 35d. Immunohistochemistry revealed increases in activated microglia in the injured dorsal horn (compared to uninjured) with the most significant increase (32%) occurring 14d post-injection. Naive and un-conjugated IB4 injected rats displayed no changes in nociceptive thresholds and insignificant levels of spinal cord CD68-IR. **Conclusion:** Selective ablation of IB4+ nociceptors triggers microglial activation coinciding with a decrease in (previously increased) nociceptive thresholds. These results imply that microglial activation contributes, in part, to the return of nociceptive behaviours reported after IB4-SAP injection despite persistent IB4+ nociceptor loss.

POS-TUE-072

SPINAL PROJECTIONS OF MID-SIZED SENSORY NEURONS EXPRESSING CALCITONIN GENE-RELATED PEPTIDE WITHOUT SUBSTANCE P IN MICE

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Purpose: Many small diameter nociceptive neurons in dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). In mouse DRG some mid-sized neurons express CGRP without SP. The projections and functions of these neurons are unknown. Therefore, we have combined in vitro axonal tracing with multiple-labelling immunohistochemistry to map their projections to the cervical spinal cord. **Methods:** The spinal cord and intact brachial plexus was removed from (C57/Bl6) mice anaesthetised in isofluorane. Neurobiotin (NB) was applied to C7 ventral ramus and incubated for 4 hours. NB was detected with streptavidin-DTAF in spinal cord and DRG sections, labelled for CGRP, SP and VGluT1 as a marker for myelinated mechanoreceptors. **Results:** 44% of CGRP neurons in DRG lacked SP with a mean soma size of 522±26µm^2 (n=4). Within cervical spinal cord, CGRP terminals lacking SP were most prominent in lateral areas of lamina I and in lamina IV. NB filled large myelinated fibres extending to the ventral horn and smaller diameter fibres that penetrated lamina IV. 7±3% (n=4) of the filled fibres penetrating lamina IV contained CGRP and 21±2% contained VGluT1 (n=2). Very few fibres were filled in lamina I and only 5±2% (n=4) and 2±1% (n=3) contained CGRP in the medial and lateral areas, respectively. **Conclusions:** Neurons containing CGRP without SP projecting to lamina IV may represent a subpopulation of polymodal mechanoceptors.

POS-TUE-073

THE EXPRESSION OF AXON GUIDANCE GENES DURING DEVELOPMENT OF THE PRIMATE MACULA

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Purpose: The macula is a highly specialised region in primate retina that mediates high spatial acuity and colour vision in the central region of the visual field. During development, macular ganglion cells extend their axons toward the optic nerve head and map onto higher visual targets. Overlapping with this axon pathfinding is a period of retinal vascular growth whereby large vessels avoid the macula. Despite its functional importance, little is known about the genes that regulate the phases of axon guidance and vascular growth with respect to the macula. We aimed to identify genes involved with these phases of growth. **Methods:** A microarray analysis was performed, using human foetal retinas at 19-20 weeks' gestation (n=4), to identify genes differentially expressed in the developing macula. Gene expression results were confirmed by quantitative RT-PCR and *in situ* hybridisation, using macaque retinas aged between foetal day 55 and adulthood. **Results:** A cluster of 25 axon guidance genes featured prominently in the microarray data and show significant differential expression between the macula and surrounding retina. Of these genes, EphA6, Unc5D and NetrinG1 are highly expressed in the macula and show similar levels of expression during formation of the avascular area at the central-most fovea. The expression of these genes changes in the postnatal period. **Conclusion:** The up-regulation of these axon guidance genes in the macula suggests they influence guidance of macular ganglion cells. We propose that EphA6 also regulates vascular patterning by inhibiting vessel growth into the fovea.

POS-TUE-074

ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION ENHANCES EXCITABILITY OF THE MOTOR CORTEX AND MOTOR FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Purpose: to review the published literature on the efficacy of anodal transcranial direct current stimulation (tDCS) on cortical excitability and motor function in both healthy individuals and subjects with stroke. We also aim to look at the tDCS parameters such as density of applied current and duration for optimal effects. **Methods**: The electronic databases (Ovid Medline, CINAHL, EMBASE, Scopus, Pubmed and CHOCHRANE) were searched for the relevant key words. Studies meeting the inclusion criteria were assessed and methodological quality was examined using PEDro and D&B assessment tools. **Results**: Data from seven studies revealed an increase in cortical excitability with a small but significant effect size (SMD: 0.31 [0.14, 0.48], p=0.0003) in healthy individuals. Data from two studies in subjects with stroke also indicated moderate significant effect size; (SMD: 0.59 [0.24, 0.93], p=0.001) in favor of anodal tDCS. Four studies examining motor function demonstrated a small non-significant effect (SMD: 0.42 [-0.14, 0.97], p=0.14) in favor of improvement in motor function in subjects with stroke. We also found that longer durations of tDCS applications and higher densities of current have larger effect sizes in favor of experiments. **Conclusions**: Anodal tDCS increases cortical excitability in both healthy individuals and subjects with stroke. The results also show a trend in favor of motor function improvement. Usually motor recovery following stroke is associated with the enhancement of brain excitability, therefore anodal tDCS could be used as a standalone or as an adds-on approach especially when survivors cannot move paralyzed limbs. Further research is needed to confirm optimal stimulation parameters for anodal tDCS. **Keywords**: transcranial direct current stimulation (tDCS), cortical excitability, motor function.

POS-TUE-075

DEVELOPMENTAL CHANGES IN INTRINSIC ELECTROPHYSIOLOGICAL PARAMETERS OF NEONATAL MOUSE HYPOGLOSSAL MOTOR NEURONS

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Purpose: Rhythmic bursts of activity in hypoglossal (XII) nerve are often used as an index of respiratory motor activity and neuromodulation in rhythmically active brainstem slices from neonatal rodents at postnatal day (P) 1 to P4. We aimed to investigate whether basic intrinsic electrophysiological parameters of mouse XII motor neurons (MNs) changed in the first 4 postnatal days. **Methods:** Whole cell patch clamp recordings were made from 41 visually identified XII MNs in transverse (200 micron thickness) brainstem slices from P1-P4 (n = 6, 13, 10 and 12 XII MNs for P1 to P4 respectively) CD1 mice anaesthetized by hypothermia or sodium pentobarbitone (100 mg/kg i.p.). Resting membrane potential, input resistance, firing rate, rheobase current and action potentials were measured. **Results:** Resting membrane potential declined (-1.6 mV/day) and steady-state firing rate increased (0.016 Hz/pA/day) linearly and significantly ($P<0.05$) from P1 to P4. Input resistance (-39 MOhm/day), action potential amplitude (-6.7 mV/day) and rheobase current (-79 pA/day) decreased linearly from P1 to P3 and then increased at P4. Action potential half-width increased (0.61 ms/day) linearly and significantly ($P<0.05$) from P1 to P3 and then decreased again at P4. Action potential after-hyperpolarization amplitude showed no consistent changes from P1 to P4. **Conclusions:** Most intrinsic electrophysiological parameters show consistent linear changes from P1 to P3 in mouse XII MNs, but many of these parameters change in the opposite direction at P4. These developmental changes will alter the excitability of XII MNs and their response to synaptic inputs and neuromodulators, highlighting the need for consideration of age in interpreting data from single XII MNs or rhythmic XII motor activity in brainstem slices from neonates.

POS-TUE-076

DELINEATION OF THE MOUSE SN, VTA, AND RETRORUBRAL FIELD USING TH, DAT, CALBINDIN, AND GIRK2

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Purpose: The substantia nigra compact part (SNC; A9), ventral tegmental area (VTA; A10), and retrorubral field (A8) are being intensively studied with gene targeting techniques in the mouse. However, there is no comprehensive analysis of these dopaminergic nuclei in the mouse. Their location has been inferred from the extrapolations from the rat. But even for the rat, there is controversy as to the border between SNC and VTA. Methods: We mapped the distribution of TH positive cells in A8, A9, and A10 in the C57BL/6J mouse (n=15). Further, we used confocal microscopy to examine the co-expression of dopamine transporter (DAT), calbindin-D-28k (Cb), and G protein-activated inward rectifier potassium channel 2 (Girk2) with TH. Results: The cellular size and disposition revealed by TH immunohistochemistry is helpful for the regional delineation of A8, A9, and A10, but a more precise delineation of these regions requires the combined information obtained from the co-expression of multiple markers with TH. The medial portion of A10 mainly contains TH^+DAT^- neurons and this distinguishes it from the parabrachial pigmental nucleus (PBP), which mainly contains TH^+DAT^+ neurons. As the major element, TH^+Cb^- neurons in the SNC highlight its border with VTA, which mainly contains TH^+Cb^+ and TH^-Cb^+ neurons. The strongest Girk2 expression is in the SNC. Conclusion: The distribution of each of these markers in relation to the distribution of TH reveals the distinct populations of neurons within A8, A9, and A10.

POS-TUE-077

DENDRITIC MORPHOLOGY OF HYPOGLOSSAL MOTONEURONS IN DEVELOPING MICE

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Purpose: The commissural crossing of dendrites and electrotonic neuronal coupling has been implicated in synchronization of firing during development of neural networks. Our aim was to study changes in dendritic trajectories and dye-coupling pattern (an indicator of electrotonic-coupling) of hypoglossal motoneurons (XII MNs) during development in mice. **Methods:** Transverse brainstem slices (300 µm thickness) were obtained from hypothermia (E18-P3) or sodium pentobarbitone (P4-P25; 100 mg/kg i.p.) anesthetized C57/Bl6 mice. Visualized XII MNs were filled with Neurobiotin™ (NB) using semi-loose seal electroporation or whole-cell patch-clamp techniques. Cells were allowed >10 minutes post-injection time for transport of NB. Subsequently, slices were fixed in 4% paraformaldehyde for 20 minutes, washed in PBS, and incubated in Cy3-streptavidin (4-8 hours). Cells were imaged with a Zeiss LSM 510 Meta confocal microscope. **Results:** A total of 62 XII MNs were filled with NB and 14 of those had dendrites projecting to the contralateral side. Cells with midline crossing dendrites distributed uniformly for all ages between E18 and P25. As evidenced by NB spread to XII MNs other than the filled cell, dye-coupling was seen in 42% (26/62) of filled cells, but significantly declined with maturation from 64% (14/22 cells) at E18-P5 to 21 % (5/24 cells) at P14-P25. **Conclusion:** Midline crossing of XII MN dendrites is established by E18 prior to birth and remains similar throughout neonatal development. Electrotonic coupling is also well established prior to birth, but declines strongly with postnatal maturation. These results suggest that dendritic projection pattern and dye-coupling patterns are differentially regulated during motoneuron development in mice. The persistence of dendritic midline crossings between E18 and P25 differs from changes reported for the rat.

POS-TUE-078

ASSESSMENT OF FUNCTIONAL DEFICITS IN SOD1-G93A MICE AND THEIR MODERATION BY EXERCISE AND METALLOTHIONEIN-IIA

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the degeneration and death of motorneurons in the spinal cord. Current therapies are mostly palliative in nature, with only one drug extending lifespan by a matter of months. Thus a more effective treatment for ALS is required. **Purpose:** To assess the progression of functional deficits in an ALS mouse model (SOD1-G93A mice) and to investigate whether these deficits may be ameliorated by exercise and/or administration of metallothionein (MT-IIA). **Methods:** Body weight, gait pattern, grip duration of SOD1-G93A (n=13) and wild-type (WT) mice (n=8) were compared. Additional groups of SOD1-G93A mice (n= 5/group) underwent a treadmill exercise program (30 min/day) with/without intramuscular MT-IIA injections (10µg/10g body weight) for 10 weeks. **Results:** SOD1-G93A mice achieved maximum weight of 20g at 11 weeks but showed decrease from 17 weeks. WT mice showed continuous weight increase, achieving 22g at 17 weeks. Grip strength compared to the WT decreased in the SOD1 mice from 14 weeks of age. By 18 weeks, none of the SOD1-G93A mice (in contrast to 90% of WT mice) were able to maintain grip for 60 seconds while inverted. By about 19 weeks, SOD1-G93A and WT mice demonstrated average step alternation measurements of 1.05 cm and 0.31 cm respectively, and stride lengths of 4.27 cm and 6.08 cm respectively. Preliminary data suggest that MT treatment with or without exercise may be able to limit the decrease in stride lengths of SOD1-G93A mice. **Conclusion:** At about 12 weeks of age SOD1-G93A mice begin to show functional deficits; MT may have a moderating effect on some deficits.

POS-TUE-079

MONOSYNAPTIC PROJECTIONS FROM THE PRECUNEIFORM AREA TO THE SPINAL CORD OF THE MOUSE

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Aims: The precuneiform area has not been demonstrated to project to the spinal cord in any of the species studied. The aim of the present study is to determine whether the precuneiform area projects to the mouse spinal cord and if so, how its fibers are distributed. **Methods:** Fluoro-gold was injected to different segments of the spinal cord and the precuneiform area was examined for retrogradely labeled cells through immunohistochemical stain of Fluoro-gold. Fluorescein or Texas red conjugated dextran amine was injected to the precuneiform area and the spinal cord was examined for anterogradely labeled fibers. **Results:** Labeled neurons were found in the precuneiform area after Fluoro-gold injections in the upper cervical (5 mice) and upper thoracic (5 mice) spinal cord, but not after upper lumbar cord (5 mice) injections. These labeled neurons were located in the medial portion of the precuneiform area. However, fewer neurons were labeled after upper thoracic injections of Fluoro-gold than cervical injections. In 6 mice, Fluorescein or Texas red conjugated dextran amine labeled fibers from the precuneiform area travelled in the ventral and lateral funiculi and terminated mainly in the medial portion of laminae 7, 8, and 9, and area 10. The density of labeled fibers tapered more caudally. **Conclusion:** The precuneiform area has monosynaptic connections with the cervical and upper thoracic cord. The precuneiform area might be part of the mesencephalic locomotor region, playing a role in head and upper limb movement.

POS-TUE-080

THE SPATIAL ORDER AND BEHAVIOR OF NEURAL CREST-DERIVED CELLS AS THE COLONIZE THE GUT

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The neural crest-derived cells that colonize the developing gut probably migrate further than any cell population in the developing embryo. Purpose: To examine the spatial order and migratory behaviour of individual enteric neural crest-derived cells (ENCCs). Methods: Time-lapse imaging of individual or small groups of cells (n = 250 cells) was performed using gut explants from embryonic mice in which ENCCs express the photoconvertible protein, KikGR. Results: The speed at which individual ENCCs migrated did not vary in the first 750 µm behind the most caudal cell, but the directionality of ENCCs in the first 150 µm was lower than ENCCs 150-600 µm behind the most caudal cell. There is therefore local leap-frogging. ENCCs migrated in close association with neurites, which appeared to act as substrates. Almost all ENCCs were motile, even in gut regions that had been colonized for over 24 hours. Conclusions: It had been assumed that the gut is colonized along its length by sub-populations of cells ceasing to migrate at each point. Our data suggest that the gut is colonized by sub-populations of cells ceasing to show directional migration. The retained migratory ability is likely to be important because the gut grows considerably after it is colonized by ENCCs. Neurites appear to be important for ENCC migration by acting as substrates, and also for maintaining the stability of the network. The migratory behaviour of ENCCs shows significant differences from that of cranial and trunk neural crest cells.

POS-TUE-081

EFFECTS OF LOCAL SOLUTION EXCHANGE ON ATP EVOKED RELEASE OF 5-HT IN GUINEA PIG ILEUM

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Purpose: Local concentrations of 5-HT at enteric sensory neuron endings in the intestine are related to the amount of 5-HT released by enterochromaffin (EC) cells, re-uptake of 5-HT and diffusion into the bath. We aimed to determine the contribution of local solution exchange on ATP evoked 5-HT concentrations at the mucosal surface. **Methods:** Ileum was taken from guinea pigs (n=11) and small silicone rings (inner diameter=1.6mm) were used to isolate areas of mucosa. 5-HT concentrations near the mucosal surface were measured electrochemically during steady state (SS) and compression-evoked (peak) release in superfused areas and in areas isolated from flow by the silicone ring. **Results:** Four spots from each preparation were compared with superfused areas showing peak 5-HT of 19±3μM and SS 5-HT of 5±1μM. Individual areas isolated from flow for 5 minutes showed a significant increase in SS levels (14±5μM, $P<0.05$, n=4). Application of ATP (100μM) to the whole bath showed peak 5-HT release of 118% of control and SS of 125% (n=3) while ATP applied to isolated areas showed peak 5-HT of 72% and SS of 81% (n=11). In the presence of the P2 receptor antagonist PPADS (60μM), peak 5-HT was 53% and SS was 32%. **Conclusion:** Our data shows that the local flow of solution acts to carry away a substantial amount of 5-HT in the *in vitro* situation. This 5-HT accumulation near the mucosal surface may play an important role in reducing further ATP-evoked 5-HT release suggesting that an *in vivo* situation with limited perfusion may reduce EC cell function.

POS-TUE-082

HEATING AND EATING: ULTRADIAN FACULTATIVE BROWN ADIPOSE TISSUE THERMOGENESIS PRECEDES BOTH FOOD INGESTION AND FOOD-SEEKING BEHAVIOUR

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Purpose: In Sprague-Dawley rats, brown adipose tissue (BAT) temperature increases in an ultradian fashion approximately every 80-100 min during the dark active phase of the circadian cycle. Variation of body temperature with eating has been noted by many investigators. The present study examined the relationship between BAT temperature increases and eating or touching the food container in rats with ad libitum access to food, as well as in rats deprived of food for up to 24 hours. Lights were on/off in 12 hour cycles. **Methods**: BAT, brain and pre-tracheal temperature were measured at 1 Hz with implanted thermistor probes (1). Eating was measured with a strain gauge coupled to the food container.**Results** Increases in BAT temperature, highly correlated with brain and body temperature, occurred in an ultradian fashion, with dark period interpeak interval of 91±49 min (mean±SD, 159 intervals in 12 rats) and light period interpeak interval of 173±98 min (mean±SD, 55 intervals). Eating occurred 15±1 min (mean±SEM, 215 episodes in 12 rats) after the onset of an increase in BAT temperature, and at virtually no other time, with no dark/light phase differences. Container-touching without eating, with or without food in the container, occurred 16±1 min (mean±SEM, 90 episodes) after the onset of an episodic increase in BAT temperature. There was no relationship between amount eaten and pre- or post-prandial time from or to a BAT thermogenic episode. **Conclusion**: Eating usually occurs as a centrally programmed act, a component of the brain-generated ultradian pattern that Kleitman described as the Basic Rest-Activity Cycle (BRAC). (1) Ootsuka et al., Neuroscience 2009.

POS-TUE-083

THE ORIGIN OF INHIBITORY INPUTS TO SYMPATHETIC PREGANGLIONIC NEURONS AND THE ROSTRAL VENTROLATERAL MEDULLA

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PURPOSE: Inhibitory input regulating sympathetic outflow and vasomotor tone has been demonstrated by functional and anatomical studies of both sympathetic preganglionic neurons (SPN) and the presympathetic neurons in the RVLM. Brain sites projecting to the SPN and RVLM are well described, however the neurochemistry of these inputs is less well described. We sought to determine the origin of inhibitory inputs to the SPN and RVLM by identifying mRNA expression for three inhibitory peptides, preproenkephalin (PPE), glutamic acid decarboxylase (GAD67) and preprosomatostatin (PPS). **METHODS:** Male Sprague-Dawley rats received unilateral RVLM microinjection (n=3) or bilateral IML injections (n=4) of the retrograde tracer, Cholera toxin B (CTB). *In situ* hybridisation for PPE, GAD67 and PPS was conducted in combination with immunohistochemistry to detect CTB labelled neurons. **RESULTS:** RVLM projections arising from the periaqueductal gray and central nucleus of the amygdala were found to co-localise with the three peptide mRNAs. Both the lateral parabrachial nucleus, and lateral hypothalamic area contained PPE expressing neurons double labelled with CTB. Some RVLM projecting neurons of the kolliker fuse nucleus contained GAD67 mRNA. Of the areas projecting to SPN, up to 60% of bulbospinal neurons in the raphe pallidus contained PPE, 39% contained GAD and 16% contained PPS. Within the raphe obscurus, 16% of CTB-labelled neurons expressed PPE, 24% expressed GAD67 and 5% expressed PPS. Of the SPN-projecting cells in the raphe magnus, 11% showed PPE expression, 55% showed GAD67 and 11% showed PPS. The A5 region also contained all three peptides in spinally projecting neurons, with 57% expressing PPE, 40% containing GAD67 and 24% containing PPS mRNA. Within the PVN, 19% of bulbospinal neurons were identified to contain PPE mRNA. **CONCLUSION:** By identifying the sites of inhibitory input to the RVLM and SPN, the contribution of inhibitory peptides to regulating sympathetic outflow and vasomotor tone can be determined.

POS-TUE-084

COMT MRNA AND PROTEIN EXPRESSION IN THE PAG AS PREDICTORS OF DISABILITY AFTER NEUROPATHIC INJURY

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The periaqueductal grey region (PAG) is critical for the expression of emotional coping behaviours. In a rat model of neuropathic pain catechol-o-methyl transferase (COMT) mRNA is upregulated in the PAG of a group of rats with persistent changes in complex behaviours identical to those seen in neuropathic pain patients. The failure to cope with neuropathic injuries can be predicted by a rats intrinsic coping style. It is possible to categorise rats prior to injury, as *predicted Persistent Disability* (pPD) or *predicted No Disability* (pND). We have reported previously, increased COMT mRNA relative to generic controls, rather than rats predisposed to developing disabilities. It is possible therefore, that our data under/over-estimated injury related COMT changes. In these experiments, COMT mRNA and protein expression were therefore compared for the PAG of pPD and pND rats (N=45) and Persistent Disability (PD) and No Disability (ND) rats (N=51). RT-PCR revealed significantly increased COMT mRNA in pPD vs pND rats ($P<0.01$). In contrast, Western blots showed no significant differences in COMT protein levels in pPD vs pND rats. Following nerve injury, COMT protein levels significantly increased in PD and ND rats, however mRNA expression levels did not differ. PD rats had significantly more COMT protein compared to pPD rats and ND rats had significantly more COMT protein than pND rats, mRNA levels did not differ. It appears that higher COMT mRNA levels in the PAG identifies rats predisposed to disability, and that protein translation occurs following injury; rats that do not develop disabilities have low COMT mRNA levels which up-regulate following injury, but result in little additional protein translation.

POS-TUE-085

VARIABILITY IN HEART RATE AND BLOOD PRESSURE IN SUBJECTS WITH PARKINSON'S DISEASE

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Many studies have utilised heart rate variability (HRV) to assess autonomic function in Parkinson's disease (PD). The problem with HRV is that there is controversy in regards to whether the low frequency (LF) component represents only sympathetic activity, or both sympathetic and parasympathetic activity. Blood pressure variability (BPV), however, has the advantage that systemic blood vessels are only innervated by the sympathetic nervous system, so measuring the LF component of BPV represents a marker of the changes in sympathetic vasoconstrictor outflow. Purpose: Comparison of HRV and BPV between participants with PD (n=25), extrapyramidal motor slowing (EPMS; n=25), older healthy controls (n=28), and young healthy controls (n=19), in order to ascertain if either of these measures can be used as early markers of non-motor symptoms in PD, as these can often precede diagnosis. Methods: Heart rate variability and blood pressure variability were assessed at rest and during two minutes of slow deep breathing. Low-frequency, high-frequency, and the low frequency/high frequency (LF/HF) ratio were measured. Results: Significant differences were only seen between the young healthy controls and the three older groups. For HRV this was seen at rest and during two minutes of slow deep breathing, whereas for BPV this was only seen during two minutes of slow deep breathing. Conclusion: As no differences between participants with PD, EPMS or age-matched controls were observed, the idea that HRV or BPV can be used as indicators of autonomic dysfunction in PD is not supported. It appears that changes in HRV and BPV seen in PD may be due to normal ageing processes rather than the disease itself.

POSTUE-086

PROTEIN KINASE A INHIBITION DEPRESSES RESPIRATORY DRIVE BUT NOT RHYTHM IN CENTRAL RESPIRATORY NETWORKS OF ADULT RAT

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Purpose: Little is known about signal transduction cascades that shape the excitability of respiratory neurons that regulate respiratory motor pattern and rhythm. We determined the respiratory effects of blocking the cAMP-protein kinase A (PKA) pathway in functional subnuclei of ventral respiratory column (VRC). **Methods:** Microinjection of Rp-adenosine cyclic monophosphothioate (Rp-cAMPS; 0.5, 1 and 5 nMol in 50 nl), a PKA inhibitor, were made into glutamate identified VRC subnuclei of urethane-anaesthetised (1.3g/kg ip), paralysed, vagotomised and artificially ventilated adult Sprague Dawley rats (n = 32). Phrenic and splanchnic sympathetic nerves, end tidal CO_2 and arterial pressure were recorded. **Results:** Bilateral microinjection of 5 nMol Rp-cAMPS into the preBotzinger Complex (preBotC, n=5) caused a robust depression of phrenic burst amplitude (22 ± 6%) and an increase in phrenic burst frequency (126 ± 6%). Pretreatment with strychnine (n=3), but not bicuculline (n=3) blocked the Rp-cAMPS-evoked increase in frequency. Microinjection of Rp-cAMPS in the rostral ventral respiratory group (n=4) or Botzinger Complex (n=4) also significantly depressed phrenic drive but had little effect on phrenic burst frequency. **Conclusion:** These findings reveal active pre- and post-synaptic cAMP-PKA signaling in the VRC is essential for maintaining basal respiratory drive. Secondly, cAMP-PKA signaling in the preBotC does not underpin rhythmogenesis, but does pre-synaptically regulate the release of glycine onto preBotC neurons.

POS-TUE-087

ANGIOTENSIN TYPE 1 RECEPTORS IN THE PARAVENTRICULAR NUCLEUS MEDIATE THE HYPERTHERMIA-INDUCED REFLEX REDUCTION OF RENAL BLOOD FLOW IN RATS

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Purpose: Increasing body core temperature reflexly decreases renal blood flow (RBF) and the hypothalamic paraventricular nucleus (PVN) plays an essential role in this response. Angiotensin II in the brain is involved in the cardiovascular responses to hyperthermia. Angiotensin II receptors are highly concentrated in the PVN, thus, the present study investigated whether aniotensin II in the PVN contributes to the cardiovascular responses elicited by hyperthermia. **Methods:** Anesthetised rats were microinjected bilaterally into the PVN (100nl/side) with saline (n = 5) or losartan (1 nmol/100nl) (n = 7). Body core temperature was then elevated from 37°C to 41°C and blood pressure (BP), heart rate (HR), RBF and renal vascular conductance (RVC) were monitored. In separate groups losartan (1 nmol) (n = 4) or saline (n = 4) was microinjected into the PVN but body core temperature was not elevated. **Results:** Increasing body core temperature in control rats elicited significant decreases in RBF (44%) and RVC (37%) and these effects were entirely prevented by pre-treatment with losartan ($P<0.001$). In rats in which body core temperature was not altered, losartan microinjected into the PVN had no significant effects on these variables. *Conclusion:* The results suggest that endogenous angiotensin II acts on AT1 receptors in the PVN to mediate the reduction in RBF induced by hyperthermia. This result may have ramifications for patients on angiotensin II receptor blockers and ACE inhibitors.

POS-TUE-088

DOES HYPERTENSION ALTER THE RESPONSES TO METABOLIC CHALLENGES?

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Purpose: Essential hypertension is commonly associated with higher level of sympathetic nerve activity. We sought to test whether increased sympathetic nerve activity levels would alter the response to metabolic challenge. Phosphorylation of tyrosine hydroxylase (TH) has been established as a marker for increased activity of this rate limiting enzyme of catecholamine synthesis. The aim was to determine TH phosphorylation and the signalling mechanisms involved, in the adrenal gland, in response to glucoprivation, in animal model of hypertension. **Methods:** Male spontaneously hypertensive rats (SHR) and normotensive Wistar-kyoto rats (WKY) aged over 20 weeks were administered with saline (n=6) or 2-deoxy-d-glucose (2-DG, 400mg/kg i.p, n=6). 20 minutes after administration, animals were anaesthetised and adrenal glands were isolated. Western blot analysis using highly specific antibodies against three phosphorylated serine residues of TH as well as signalling proteins was performed. **Results:** The blood pressure in SHR were significantly higher compared to WKY (1.5 fold). 20 minutes after the administration 2DG, the blood glucose was significantly higher in the animals compared to those receiving saline group (2 fold) in both WKY and SHR. In SHR following 2DG, phosphoSer40TH was significantly increased (1.5 fold) compared to saline but no change was evident in WKY. On the other hand, phosphoSer31TH was significantly increased in both SHR (3.8 fold) and WKY (3.3 fold). PhosphoSer19TH was elevated in WKY in response to 2DG (1.7 fold) but no such change was evident in SHR. **Conclusion:** Although the same metabolic response was evident in response to glucoprivation in both SHR and WKY a different pattern of phosphorylation of TH was evident in the adrenal medulla. Whether the hypertensive phenotype contributes to these differences remains to be determined.

POS-TUE-089

RESPIRATORY MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY IS NOT INCREASED IN PATIENTS WITH ESSENTIAL HYPERTENSION OR CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Purpose: It is known that muscle sympathetic nerve activity (MSNA) occurs as bursts that are coupled to the cardiac rhythm, yet are also influenced by respiration. Given the importance of the physiological coupling between the cardiovascular and respiratory systems, it is surprising that little research has assessed how strong this coupling is in different disease states. Based on findings in the spontaneously hypertensive rat, in which respiratory modulation of sympathetic vasoconstrictor drive was shown to be increased (Simms et al., 2009), we tested the hypothesis that the magnitude of respiratory modulation of MSNA is likewise increased in states of elevated MSNA in human subjects. Methods: We examined respiratory modulation of MSNA in 13 patients with essential hypertension (HT) and 15 with chronic obstructive pulmonary disease (COPD) and compared these with a group of young healthy controls (YHC) and older healthy controls (OHC). MSNA was recorded via tungsten microelectrodes inserted percutaneously into the common peroneal nerve. Respiratory modulation of MSNA was quantified by fitting smoothed polynomials to the cross-correlation histograms constructed between the sympathetic spikes and respiration. Results: Respiratory modulation in HT (45.2±5.7%) and COPD (37.5±6.3%) was not higher than in the OHC (47.2±5.4%) or YHC (49.5±6.0%) groups, despite the significantly elevated MSNA in HT and COPD. Conclusions: We have shown that respiratory modulation of MSNA is present in all groups and is not increased in HT or COPD, arguing against an amplified respiratory-sympathetic coupling in hypertension. Moreover, given that patients with COPD are chronically asphyxic, these data indicate that an increased chemical drive does not increase respiratory modulation of MSNA. Simms et al., 2009. J Physiol. 587: 597-610.

POS-TUE-090

VESTIBULAR INFLUENCES ON MUSCLE AND SKIN SYMPATHETIC NERVE ACTIVITY: LOW-FREQUENCY SINUSOIDAL GALVANIC VESTIBULAR STIMULATION REVEALS TWO PEAKS OF MODULATION

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Purpose: We have previously shown that sinusoidal galvanic vestibular stimulation (sGVS) can cause partial entrainment of sympathetic nerve activity to muscle (MSNA) and skin (SSNA) at frequencies ranging from 0.2-2.0 Hz (Grewal et al., 2009; James et al., 2009). Here we test the effect of sGVS on sympathetic outflow when stimulating the vestibular system at lower frequencies. Methods: MSNA and SSNA were recorded on separate occasions via tungsten microelectrodes inserted into the left common peroneal nerve in 12 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 100 cycles) was applied to the mastoid processes at 0.08, 0.13 and 0.18 Hz. Results: cross-correlation analysis revealed two bursts of modulation of MSNA and SSNA for each cycle of stimulation. We believe the primary peak is related to the positive phase of the sinusoid, in which the right vestibular nerve is hyperpolarised and the left vestibular nerve depolarised. Furthermore, we believe the secondary peak is related to the negative phase of sinusoid (depolarisation over the right vestibular nerve and hyperpolarisation of the left vestibular nerve). This was never observed at higher frequencies of stimulation, presumably because at such frequencies there is insufficient time for a second peak to be expressed. Conclusions: These observations emphasize the role of the vestibular apparatus in the control of blood pressure and skin blood flow, and further suggest convergence of bilateral inputs from vestibular nuclei onto the output nuclei for MSNA (rostral ventrolateral medulla) and SSNA (medullary raphe). Grewal, James & Macefield. Exp Brain Res 2009 197: 379-386 James, Stathis & Macefield. Exp Brain Res 2010 202: 291-298.

POS-TUE-091

DUAL LABELED AUTONOMIC PREMOTOR NEURONS INNERVATING THE SUBMANDIBULAR GLAND

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Purpose: Co-ordinated modulation of the sympathetic and parasympathetic nervous systems is often observed. Whilst the sympathetic and parasympathetic pre- and postganglionic pathways are separate, overlapping distributions of the antecedent premotor neurons is observed. We have examined the distribution of the premotor neurons projecting to the submandibular gland (SMG) to understand whether single premotor neurons project to, and potentially regulate both sympathetic and parasympathetic activity. **Methods**: Two isogenic strains of the Pseudorabies virus were used which differ only in the fluorescent reporter gene expressed. In each animal (n=4) the sympathetic nerve supplying the SMG was isolated and one strain of the virus microinjected into the nerve– it was then cut peripherally to sympathectomise the gland and to prevent spread of the virus. The other strain of virus was then microinjected unilaterally into the sympathectomised SMG. **Results**: Only one fluorophore was observed in either the sympathetic or parasympathetic pre- and postganglionic neurons indicating specificity of the viral labeling protocol. As previously described fluorescently-labeled neurons were observed in a characteristic distribution throughout the brain. However, many brain regions (e.g. nucleus of the solitary tract, lateral reticular nucleus and paraventricular nucleus) contained double-labeled neurons indicating that these neurons projected axon collaterals to both the sympathetic and parasympathetic pathways innervating the SMG. **Conclusion**: These dual labeled neurons have the anatomical characteristics that would enable them to co-ordinate autonomic regulation of salivary function.

POS-TUE-092

ALMS1 MUTATION LEADS TO DECREASED CILIATION OF HYPOTHALAMIC NEURONS IN OBESE *FOZ/FOZ* MICE

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Purpose: Defects in hypothalamic primary cilia have been shown to correlate closely with hyperphagic-obesity. *Alms1* mutant (*foz/foz*) mice have a cilial disorder that is strongly associated with obesity, diabetes, metabolic syndrome, and leptin resistance. In this study we investigated hypothalamic Alms1 expression and primary cilia number and structure in relation with obesity in these mice. **Methods:** Hypothalamic proteins were estimated by western blot, mRNA expression by semi-quantitative real-time PCR. *In vitro* experiments were performed in hypothalamic primary neuronal cultures derived from WT and *foz/foz* mice. We used anti-Alms1 antibodies and the cilial marker ACIII to perform immunohistofluorescence on murine hypothalamus. The number and length of primary cilia were measured at 3wk (weaning), 8 and 18wk of age (n>5). Mice (*foz/foz* or wildtype (WT)) were fed either rodent chow or high-fat diet. **Results:** While genotype, age and diet did not affect the length of hypothalamic primary cilia, a dramatic decrease in their number (~32% of WT) was found in association with decreased *Alms1* mRNA expression in *foz/foz* mice. Primary hypothalamic neuron culture of WT mice demonstrated *Alms1* express as a spherical doublet at the base of the primary cilium. This doublet appeared lost in *foz/foz* mice. **Conclusion:** These results show, for the first time, that Alms1 locates to the basal body of primary cilia on neurons involved in appetite regulation. Further, mutation of *Alms1* in *foz/foz* mice is associated with a major reduction in number of neuronal primary cilia. Since several appetite regulating molecules (MCH1R, SSTR3, leptin receptor possibly) may be selectively located on the primary cilium, this cilial defect could be pivotal to the mechanism of obesity in *foz/foz* mice.

POS-TUE-093

THE EFFECT OF DIET-INDUCED OBESITY ON KISSPEPTIN GENE EXPRESSION WITHIN THE MOUSE HYPOTHALAMUS

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The primary controllers of fertility in the brain are the gonadotrophin-releasing hormone (GnRH)-expressing neurons. Leptin is an anorexogenic hormone that relays information about body fat status to the brain. Leptin affects reproductive function, however it does not directly stimulate GnRH neurons suggesting an intermediary messenger such as the GnRH-stimulating neuropeptide kisspeptin. **Purpose**: this study aimed to measure hypothalamic kisspeptin mRNA in a state of diet-induced obesity. **Methods**: female C57BL/6J (n=20) and DBA/2J (n=23) mice were used because these different strains of mice are known to be differentially susceptible to high-fat diet (HFD) induced infertility. Half of each group was fed a 23% high-fat rodent diet and the remaining half was fed standard rodent chow for two and a half months. Mice were ovariectomised (to normalise circulating oestrogen levels) seven days before brain collection. Messenger RNA was isolated from kisspeptin-expressing areas of the hypothalamus: rostral periventricular area of the third ventricle (RP3V) and arcuate nucleus (ARC). Kisspeptin mRNA levels were investigated using quantitative RT-PCR. **Results**: DBA mice were susceptible to HFD, displaying a HFD-induced decrease in kisspeptin mRNA expression in the RP3V (24% of chow fed control levels) and ARC (42% of chow fed control levels; $p < 0.05$ and $p = 0.53$ respectively). In contrast, hypothalamic kisspeptin gene expression did not change in C57 mice fed a high fat diet. Additionally, female DBA mice had at least 10-fold lower hypothalamic kisspeptin mRNA levels than female C57 mice. **Conclusion**: this study supports the idea that kisspeptin-expressing neurons may act as an intermediate between leptin and GnRH neurons during times of diet-induced obesity, and that this system becomes leptin resistant in DBA mice.

POS-TUE-094

SYMPATHETIC AND RESPIRATORY CONTROL BY HYPOTHALAMIC PERIFORNICAL AREA: CORRELATION WITH OREXIN NEURON DISTRIBUTION

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Neurons within the hypothalamic perifornical area (PeF), particularly orexin (hypocretin) neurons, may have a critical role in generating cardiorespiratory responses associated with arousal or exercise. In this study we mapped sites within the PeF and surrounding regions at which neuronal activation produced marked increases in sympathetic and/or respiratory activity, and correlated this with the density of orexin neurons within the same locations. The results were also correlated with the distribution of neurons that expressed Fos (a marker of neuronal activation) following a period of voluntary exercise in conscious rats, using data from a previous study (1). Renal sympathetic nerve activity (RSNA) and phrenic nerve activity (PNA) were recorded in rats (n=11) anaesthetized with urethane. Microinjections of bicuculline (10 pmol in 20 nl) evoked large increases in PNA burst rate and RSNA from sites within the PeF, mainly within the region extending from 2.4-3.5 mm caudal to bregma. Average increases in PNA burst rate and RSNA evoked from the PeF (53 ± 6% and 41 ± 4% baseline, respectively) were significantly greater in both cases ($P < 0.05$) than responses evoked from the regions immediately dorsal or ventral to the PeF. This responsive region contains a high density of orexin neurons and also of neurons that expressed Fos following a period of voluntary exercise. The results demonstrate that neurons within the PeF are capable of generating potent sympathoexcitatory and respiratory responses, and are consistent with the hypothesis that orexin neurons in this region contribute to such responses during exercise. 1) Lam ACB et al., Proc Aust Neurosci Soc 20:98, 2010.

POS-TUE-095

HIPPOCAMPAL VOLUME IS DECREASED IN HUMANS AND RATS WITH NEUROPATHIC PAIN

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Following sciatic nerve chronic constriction injury, rats that display alterations in social behaviour, disrupted sleep-wake cycle, and endocrine dysregulation, as well as pain, have a significantly smaller volume of the hippocampal dentate gyrus, when compared with rats that have pain alone. Our observations are consistent with a number of studies, which show that other physical and/or psychological stressors are associated with significant changes in the structure of the hippocampus. To determine whether pain associated with nerve damage in humans is associated with hippocampal volume changes, super-sampled, non-morphed, T2 weighted MRI scans from people with trigeminal neuropathic pain (TNP; N=10) were compared to scans of individuals with temporomandibular disorder (TMD; N=8) a non-neuropathic, musculoskeletal orofacial pain condition, as well as healthy age and sex matched controls (C; N=18). The TNP and TMD patients reported similar intensities and durations of pain. The TNP subjects had significantly smaller hippocampal volumes compared with both, healthy controls (C); and people with temporomandibular disorder (TMD) ($p<0.05$; Mann-Whitney U). These observations reveal the rat sciatic nerve chronic constriction injury as a valuable model to investigate nerve-injury induced hippocampal atrophy, in particular the underlying pathophysiological changes and their functional consequences. The data also suggest that structural, as well as functional changes in higher brain regions, likely play a role in the expression of pain and disability triggered by nerve damage.

POS-TUE-096

AXOTOMY OF SACRAL PREGANGLIONIC NEURONS TRANSIENTLY UPREGULATES C-JUN AND ATF-3, AND DOWNREGULATES CHOLINE ACETYLTRANSFERASE EXPRESSION IN A SUBSET OF INJURED NEURONS

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Purpose: Understanding neuronal changes after nerve injury or axotomy, e.g. in cauda equina syndrome or after abdominal surgery, is crucial to development of regenerative strategies. Postganglionic axotomy or deafferentation upregulates c-Jun, an injury- and regeneration-associated immediate early gene, in pelvic ganglion neurons and glia. In this study we examined the effects of axotomy on preganglionic neurons from lumbar and sacral spinal cord. **Methods:** In adult male Wistar rats, we identified preganglionic neurons using a retrograde tracer (FluoroGold; 300 µl, 0.5%, i.p). Pelvic or hypogastric nerves were unilaterally transected (n≥4 per group) and effects were assessed immunocytochemically at one, two, and four weeks after injury. Expression of c-Jun, the neuronal injury marker activating transcription factor-3 (ATF-3), and choline acetyltransferase (ChAT) were examined. **Results:** The effects of injury were greatest in sacral cord, ipsilateral to pelvic nerve transection. One week after injury, ipsilateral c-Jun and ATF-3 expression were upregulated: >30% of ChAT neurons expressed c-Jun, and ~10% expressed ATF-3. Conversely, ~25% of c-Jun neurons and ~60% of ATF-3 neurons were ChAT-negative, and the remaining ATF-3 neurons were weakly ChAT-positive. Soma size and overall intensity of ipsilateral ChAT labelling were also reduced ipsilateral to injury. In naive controls, <10% of ChAT neurons expressed c-Jun, none expressed ATF-3, and ChAT-negative retrogradely labelled neurons were rare. **Conclusion:** A significant proportion of axotomised sacral preganglionic neurons transiently downregulate ChAT, and upregulate c-Jun and/or ATF-3 expression. These effects were limited to a subset of injured neurons, whereas uninjured spinal levels, neighbouring interneurons, and glia were unaffected.

POS-TUE-097

INDUCTION OF FOS IMMUNOREACTIVITY IN BULBOSPINAL AND NEUROCHEMICALLY CHARACTERIZED BRAIN SITES DURING GLUCOPRIVATION

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Purpose: Multiple sites in the lower brainstem (including the rostral ventrolateral medulla; RVLM) and hypothalamus are activated in response to falling glucose levels and play a key role in the generation of a complex counterregulatory response. We sought to determine the distribution, chemical phenotype and projection pattern of neurons in the brain activated following glucoprivation. **Methods:** Brain sections (40µm) from male Sprague Dawley rats sacrificed 2 hours after 2-deoxyglucose (2DG, 400mg/kg, ip) or saline administration were processed for *in situ* hybridization (neuropeptide Y (NPY), glutamic acid decarboxylase or preproenkephalin) combined with immunohistochemistry (Fos, tyrosine hydroxylase, (TH), cholera toxin B (CTB)). In three animals, the retrograde tracer CTB was injected into the upper thoracic spinal cord one week prior to glucoprivation, to target bulbospinal RVLM neurons. **Results:** Glucoprivation induced Fos expression in the VLM, dorsal vagal complex, locus coeruleus, ventromedial hypothalamus and paraventricular nucleus confirming previous findings. Previously undescribed sites also activated by glucoprivation include the amygdala (central nucleus), lateral hypothalamus, medial habenula, geniculate region and supramammilary nucleus. Our first data set demonstrates that four distinct populations of neurons are activated in the VLM in response to 2DG induced glucoprivation. Half of all bulbospinal and TH-ir neurons (restricted to RVLM) were activated by 2DG (48±1%; 83±5 TH-ir/CTB-ir/Fos-ir of 179±11 TH-ir/CTB-ir neurons, n=3). A large number of activated bulbospinal non-TH-ir neurons were also observed. Our preliminary data from RVLM indicates that virtually all activated, catecholaminergic neurons contain NPY mRNA therefore NPY is not a unique marker of bulbospinal neurons activated by glucoprivation. **Conclusion:** We hypothesise that different subpopulations of neurons represent functionally distinct pathways controlling the response to hypoglycaemic challenge.

POS-TUE-098

THE GALANIN-3 RECEPTOR ANTAGONIST, SNAP 37889, REDUCES OPERANT RESPONDING FOR ETHANOL IN ALCOHOL-PREFERRING RATS

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Purpose: The galanin-3 receptor (GALR3) subtype has been identified as having a role in alcohol consumption and general feeding behaviours. The present study investigated the potential of the novel selective GALR3 antagonist, SNAP 37889, to reduce voluntary ethanol consumption in the iP (alcohol-preferring) rat. **Methods:** To examine the effect of SNAP 37889 treatment on ethanol consumption and reward-seeking behaviour, iP rats (n=12 per group) were trained to lever press for water and solutions containing either 10% (v/v) ethanol, 5% (w/v) sucrose or 0.1% (w/v) saccharin as part of an operant paradigm. Once a base level of responding was established, rats were pre-treated with SNAP 37889 (30mg/kg, i.p.) or vehicle and the number of rewards made during operant sessions was recorded. In addition, testing of rat locomotor activity was tested to determine whether SNAP 37889 had a sedative effect at the dose used in this study. **Results:** Overall, SNAP 37889 (30 mg/kg, i.p.), significantly reduced operant responding for solutions containing ethanol, sucrose and saccharin. Moreover, SNAP 37889 did not alter locomotor activity at the dose administered in this study. Collectively, results from the current study show that SNAP 37889 (30 mg/kg, i.p.) is effective in reducing operant responding for ethanol, independent of a sedative effect. **Conclusions:** These findings provide evidence that GALR3 antagonism reduces alcohol consumption and further suggest that GALR3 may be implicated in the rewarding effects of natural and drug reinforcers.

POS-TUE-099

ALTERED DNA METHYLATION IN THE MEDIAL PREFRONTAL CORTEX FOLLOWING COCAINE SELF-ADMINISTRATION AND PASSIVE WITHDRAWAL

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The development of cocaine addiction is characterized by lasting changes in brain reward circuitry, which leads to an increase in susceptibility to relapse during drug abstinence. This susceptibility is exacerbated by a time-dependent intensification of cue-induced cocaine cravings during withdrawal, through a process known as incubation of cocaine craving. Epigenetic modifications, such as DNA methylation, have been shown to regulate experience-dependent changes in gene expression incurred by cocaine self-administration training; however, whether these learning-induced alterations in DNA methylation persist and contribute to the incubation of cocaine craving has not been investigated. In this study, we have used an epigenome-wide profiling approach (methylated DNA immunoprecipitation followed by microarray) to determine whether altered DNA methylation patterns persist in the ventral medial prefrontal cortex (vmPFC) following cocaine self-administration and 30 days of passive withdrawal. Subsequent to cocaine self-administration and passive withdrawal, we detected a significant decrease in DNA methylation within 288 gene promoters in cocaine-treated mice (n=7) relative to naïve controls (n=5). Persistent changes in DNA methylation may thus contribute to the metaplastic changes in gene expression that occur as a function of cocaine-seeking behaviour and are associated with the incubation of cocaine craving.

POS-TUE-100

EFFECT OF OXYTOCIN ADMINISTRATION IN THE NUCLEUS ACCUMBENS CORE OR THE SUBTHALAMIC NUCLEUS ON METHAMPHETAMINE-INDUCED REWARD

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PURPOSE: The neuropeptide oxytocin has been implicated in modulating the rewarding effects of methamphetamine (METH), however, little is known about the neurobiological mechanisms acutely involved. Recent findings have suggested that oxytocin may be attenuating METH-related reward within 2 substrates, namely the nucleus accumbens (NAc) core and subthalamic nucleus (STh). We aimed to investigate the effect of a pretreatment of oxytocin (0.6 ng) directly within the NAc core and STh on METH (1 mg/kg) single trial conditioned place preference (CPP) in male Sprague Dawley rats (n=84). **METHODS:** Rats underwent surgery for the implantation of bilateral microinjection cannulae (26 GA) in the NAc core or STh under isoflourane anaesthesia. After a recovery period of 5 -7 days, CPP was conducted using 2 daily conditioning sessions (30 min), where drug treatment was paired with 1 context, and vehicle administration was paired with the other context. Odour cues were used to differentiate the 2 contexts. **RESULTS**: Our results showed that a single trial METH CPP increased the time rats spent in the METH-paired context and decreased the time spent in the saline-paired context across testing days, indicating a CPP to METH ($p < .005$). A single trial CPP with a pretreatment of oxytocin into the NAc core ($p < .0005$) or STh ($p < .05$) prior to a single METH treatment prevented METH-induced CPP. **CONCLUSION:** These findings show that oxytocin reduces the rewarding experience of METH through modulation of the NAc core and STh.

POS-TUE-101

GENE-ENVIRONMENT INTERACTIONS MODULATING SCHIZOPHRENIA ENDOPHENOTYPES IN THE METABOTROPIC GLUTAMATE RECEPTOR 5 KNOCKOUT MOUSE

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The role of glutamatergic signalling in psychiatric illness, the development of which results from a complex interplay between genetic and environmental factors, is well recognized. Metabotropic glutamate receptor 5 (mGluR5) has been associated with schizophrenia and mGluR5 knockout (KO) mice have been shown to exhibit behavioural abnormalities regarded as endophenotypes of relevance to schizophrenia. To investigate gene-environment interactions within the mGluR5 KO mouse, mice were housed in standard or environmentally enriched conditions and their performance assessed in a number of behavioural tests. We have demonstrated that enrichment can improve cognition in the Morris water maze, ameliorate hyperactivity and rescue PPI deficits in KO mice. Previous studies have shown that KO mice have increased sensitivity to the hyperlocomotive effects of the psychomimetic MK-801, a NMDA receptor antagonist (Gray et al., Int J Neuropsychopharmacol 2009). Enriched and standard housed mice were tested after administration of 0.15mg/kg and 0.25mg/kg doses of MK-801. As expected, wild-type (WT) mice exhibited increased locomotion on administration of the high dose only. A heightened response was seen in KO mice, with both low and high doses of MK-801 inducing hyperactivity. Interestingly, KO mice exposed to environmental enrichment exhibited even greater levels of hyperactivity following MK-801 treatment compared to standard-housed controls. Enrichment had no effect on the response to MK-801 in WT mice. Reduced PPI in KO mice was resistant to further reduction by MK-801 treatment. Environmental enrichment reinstated MK-801-induced PPI disruption in KO mice. Contrary to the effects in KO mice, enrichment reduced the disruptive effect of the antagonist on PPI in WT mice. These results demonstrate that behavioural abnormalities relevant to schizophrenia exist in the mGluR5 KO mouse and are selectively modulated by enrichment. Furthermore, they highlight the interactions between mGluR5 and NMDA receptors in the determination of schizophrenia-like behaviours.

POS-TUE-102

A DISSOCIABLE ROLE FOR HISTONE ACETYLTRANSFERASES P300/CBP AND PCAF IN THE EXTINCTION OF CONDITIONED FEAR

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The histone acetyltransferases (HATs) p300 and CREB-binding protein (CBP) are essential for the formation of contextual fear memory (Vescey et al., 2007, Journal of Neuroscience, 27, 6128-6140). Additionally, the HAT co-activator, p300/CBP-associated factor (PCAF), is also important for learning and memory. Mice with a global deletion of PCAF show exaggerated stress reactivity, enhanced contextual fear and impaired reversal learning (Maurice et al., 2008, Neuropsychopharmacology, 33, 1584-1602). Given these observations, we hypothesized that individual epigenetic regulatory proteins may have distinct roles associated with different emotional memory processes. We infused antagonists specific for PCAF, p300/CBP or p300 directly into the ventromedial prefrontal cortex (vmPFC) immediately after extinction training. Memory for the extinction of conditioned fear was impaired in mice infused with the PCAF antagonist, whereas mice infused with either a p300/CBP or p300 antagonist into the same brain region showed enhanced fear extinction memory. These data suggest that individual HATs make unique contributions to memory formation with PCAF being necessary, and p300/CBP and p300 being dispensable, for fear extinction.

POS-TUE-103

THE ROLE OF DOPAMINE AND NORADRENALINE RECEPTORS IN IMPULSIVITY MEDIATED BY THE MEDIAL PREFRONTAL OR ORBITOFRONTAL CORTEX IN RATS

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Purpose: Impulsivity is characteristic of many disorders, such as Attention Deficit/Hyperactivity Disorder (ADHD) and drug addiction. Impulsive choice is largely mediated by the prefrontal cortex subregions: the medial prefrontal cortex (mPFC) and the orbitofrontal cortex (OFC). Dopamine (DA) and noradrenaline (NA) are known to modulate activity of the prefrontal cortex, however their direct role in impulsive behaviour is yet to be described. The aim of the present study was to investigate the effect of microinjections of DA or NA receptor compounds in the mPFC or OFC on impulsivity as measured by a delayed reinforcement task (DRT) in rats. **Methods:** Male Wistar Kyoto rats (n=59) underwent surgery for the implantation of bilateral microinjection cannulae (26 Ga) into the mPFC or OFC under isofluorane anaesthesia. Following 5-7 days of recovery, rats were trained in operant chambers for the DRT to associate one lever with a single food pellet (immediate reward) or the other with five food pellets after a time delay. Rats were pretreated with microinjections of DA D1 and D2 receptor antagonists (SCH23390 3 µg/side, Raclopride 3 or 6 µg/side) or NA α1 and α2 receptor agonists (Phenylephrine 0.1 or 0.3 µg/side, Guanfacine 1 or 3 µg/side) into the mPFC or OFC and the effect on impulsive behaviour was assessed. **Results:** Pretreatment with SCH23390 significantly increased impulsivity in the mPFC ($P<0.05$) but not OFC, as did raclopride for both brain regions ($P<0.05$). Pretreatment with the α receptor agonists had no effect on impulsivity in either brain region. **Conclusion:** This study suggests that DA receptors, but not α (NA) receptors, differentially mediate impulsivity in subregions of the prefrontal cortex.

POS-TUE-104

PRENATAL RESTRAINT STRESS LEADS TO EPIGENETIC MODIFICATIONS IN THE VENTROMEDIAL PREFRONTAL CORTEX IN ADULT OFFSPRING

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Environmental factors such as nutrition, maternal care and stress have a profound impact upon developmental processes during early life, which in turn is associated with future disease susceptibility. Prenatal stress (PNS) leads to increased susceptibility to anxiety-related behavioural phenotypes such as post-traumatic stress disorder and depression, thus suggesting that the early life environment affects development processes within the brain. In this study, employing a mouse model, and specifically targeting a region of the brain that plays an important role in emotional learning and memory (i.e. the ventromedial prefrontal cortex; vmPFC), we sought to determine whether epigenetic mechanisms are involved in these phenomena. The effects of maternal restraint stress during the final week of pregnancy were assessed in male mice from two genetic backgrounds: (1) stress resilient DBA/2J (D2), and (2) stress vulnerable C57/Bl6 (B6). Genome wide DNA methylation profiles were determined in adult offspring (9 weeks) by enriching for methylated DNA and hybridizing to promoter CpG island microarrays (n=5 per group). PNS led to increased reward-seeking behaviour in adult offspring, and this was associated with DNA methylation differences at numerous gene promoters within the vmPFC. Many of these genes are known to be involved in plasticity-related processes (e.g. Cacna1b). These results strongly suggest that epigenetic mechanisms within the vmPFC are associated with PNS-mediated behavioural outcomes.

POS-TUE-105

THE HISTONE DEACETYLASE RPD3 IS REQUIRED FOR LONG-TERM COURTSHIP MEMORY IN DROSOPHILA MELANOGASTER

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Purpose: There is increasing evidence that regulation of local chromatin structure is a critical mechanism underlying the consolidation of long-term memories. To this end, we are investigating the role of histone acetylation in the storage of long-term memories (LTM) in Drosophila. Given that the core molecular pathways required for memory are conserved across invertebrates and vertebrates, Drosophila is an ideal model for studying memory due to the myriad genetic tools and reproducible memory assays that have been developed. Rpd3 is a Class I histone deacetylase, with approximately equal homology to human HDAC1 and HDAC2. Rpd3 is expressed ubiquitously in neuronal nuclei, including the mushroom body (MB), which is the predicted site of memory formation and storage. **Methods:** To assess whether Rpd3 is required for normal LTM, a MB-specific driver was used for RNAi-mediated knockdown of Rpd3. Memory was assessed using the repeat-training courtship conditioning assay (n=20/group). In this paradigm, wild type males remember that they have been previously rejected by a female and thus display reduced courtship behaviour when exposed to a new female. **Results:** Control males showed robust LTM ($p<0.001$), but Rpd3 knockdown males did not show significant LTM ($p=0.24$). Immunohistochemical analysis of MB structure did not reveal any obvious perturbations in Rpd3 knockdown brains, however to control for possible subtle developmental effects, Rpd3 was knocked down in adult brains using a temperature-regulated system. While control males showed normal LTM ($p<0.001$), it was not significant in Rpd3 knockdown males ($p=0.13$). **Conclusion:** Rpd3 plays a role in LTM in Drosophila. Studies to determine the mechanism by which Rpd3 affects LTM are ongoing.

POS-TUE-106

SELECTIVE OVEREXPRESSION OF Aβ42 IN THE RAT HIPPOCAMPUS LEADS TO NEURONAL CELL DEATH AND IMPAIRED NEUROGENESIS

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Purpose: The abnormal accumulation of amyloid beta (Aβ) protein and its deposition in neuritic plaques in the brain is a key pathological feature of Alzheimer's disease (AD). A system whereby Aβ peptides are expressed as fusion proteins of the human type 2 integral membrane protein (BRI) has been developed to enable elucidation of the role of specific Aβ peptides in AD pathogenesis. The aim of this study was to characterize early behavioural and histopathological changes following BRI-Aβ42 overexpression. **Methods:** Rats received an intrahippocampal infusion of recombinant adeno-associated viral (AAV) vector expressing a BRI-Aβ42 fusion protein (n=15), control AAV-BRIΔ (n=15) or PBS (n=10). Four weeks post-infusion, the effects of Aβ42 overexpression on performance in three hippocampal-dependent learning and memory tests (novel object recognition, Morris water maze and passive avoidance) was assessed and then brain tissue analysed by immunohistochemistry. **Results:** Subtle cognitive deficits were found by 4 weeks. Behavioural changes were associated with neuroinflammation, dendritic loss and neuronal degeneration within the hippocampus of BRI-Aβ42 rats but not in controls. Aβ plaque deposition had not occurred by 8 weeks post-AAV infusion. However, neurons within affected regions expressed high levels of transgene and immunohistochemically detectable non-aggregated Aβ42, supporting a role for intraneuronal or soluble extracellular Aβ42 in Aβ-mediated toxicity. Furthermore, while levels of proliferating neural precursors in the subgranular region were similar, numbers of immature neurons were significantly reduced in BRI-Aβ42 rats compared to controls (ANOVA; $p<0.05$). **Conclusions:** This model complements current transgenic rodent AD models and provides a novel system for dissecting the pathogenicity of individual Aβ peptides.

POS-TUE-107

EFFECT OF HIGH FAT AND REFINED SUGAR DIETS ON HIPPOCAMPAL DEPENDENT LONG TERM MEMORY

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Purpose: Recent research has demonstrated that a diet high in saturated fat and refined sugars results in impaired hippocampal dependent memory function in rats. While some research has demonstrated a link between diet and cognition in human populations, a specific effect of saturated fat and refined sugar diet consumption on memory has not yet been investigated. **Methods:** 490 undergraduate university students completed a dietary screener to measure dietary saturated fat and refined sugar intake, the Three Factor Eating Questionnaire (TFEQ) as well as computerised measures of Logical Memory (LM), Verbal Paired Associate Learning (VPAL) and Digit Span (DS) subtests of the Weschler Memory Scale-Revised. **Results:** When controlling for restraint (TFEQ), there was a significant negative correlation between dietary screener scores and delayed LM performance ($r= -0.10$, $p< 0.05$) as well as delayed VPAL performance ($r = -0.12$, $p < 0.01$). No significant correlation was found between dietary screener scores and immediate performance on LM and VPAL tasks or digit span. **Conclusion:** These results indicate that dietary fat and sugar intake is selectively associated with impaired performance on hippocampal long term memory tasks.

POS-TUE-108

THE LONG TERM EFFECT OF TREATMENT WITH METHYLPHENIDATE AND CAFFEINE THROUGH ADOLESCENCE IN WISTAR KYOTO RATS

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Purpose: Impulsivity is a dimension of personality that can influence behaviour across a range of circumstances and situations. In both rats and humans, impulsive choice is measured using the delayed reinforcement task (DRT). The subject can choose a small immediate reward or a larger delayed one. The time delay to the larger reward can be increased to the point where a smaller immediate reward is preferred. This study investigated the effect on impulsive choice in the DRT paradigm following exposure in early adolescence to one of two psychostimulants, caffeine (CAFF) or methylphenidate (MPH) or their combination, in male Wistar-Kyoto rats. **Method:** Adolescent rats (n=48) were treated for 5 weeks with either Water, MPH 2mg/kg, CAFF 7.5mg/kg or combined MPH and CAFF. When drug free in adulthood, rats were then trained to respond on one lever to receive a single food pellet or the other to receive 3 food pellets. During test days both levers were active and increasing delays (0, 1, 3, 6, 12, 24 sec) were introduced to responses at the delayed lever for the DRT. **Results:** There was a significant effect of CAFF treated animals compared to MPH or water treated animals at the 12 sec delay ($p<0.05$). Combined treated animals were not significantly different to water or MPH, suggesting an attenuation of CAFF effects by concomittant MPH administration. **Conclusion:** The data suggest that exposure to caffeine through adolescence has the potential to change cognitive functioning in adulthood which may be moderated by MPH co-administration.

POS-TUE-109

THE EFFECTS OF CALORIE RESTRICTION ON OPERANT-RESPONDING FOR ETHANOL IN THE ALCOHOL-PREFERRING RAT

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Purpose: The aim of this study was to establish whether the anxiolytic effects of calorie restriction (CR) would reduce alcohol self-administration in alcohol-preferring (iP) rats, a strain with an anxious phenotype. **Methods:** Twenty-two iP rats were used to examine these effects, 11 of which were fed ad libitum, and the remaining administered a 25%CR for four weeks prior and throughout behavioural testing. All rats were then tested on both the elevated plus maze and in the open field prior to being trained to operantly self-administer either 10% v/v ethanol, or water. **Results:** A significant difference was found between the control and CR25% group in the percentage of open arm/total arm duration (29.07±3.87 vs 44.57±3.39, p=0.0069) and percentage of open arm/total arm entries (33.45±2.94 vs 51.36±3.28, p=0.0006) in the elevated plus maze. Both control and CR25% groups showed preference for alcohol rewards vs water, however CR25% rats responded significantly less for alcohol (14.3±0.8 vs 19.7±0.9, p=<0.0001) during the conditioning phase. A similar effect was also observed post 4 weeks forced abstinence with control rats showing a significant alcohol deprivation-effect (34.38±5.5 vs 19.8±3.4, p=0.03) while no significant difference were found in the CR25% group. However, CR25% failed to significantly alter cue-induced reinstatement of alcohol-seeking. **Conclusions:** Taken collectively, these results demonstrate that the anxiolytic effects of CR25% reduces operant responding for ethanol and inhibits an alcohol deprivation effect in iP rats. Therefore, CR may potentially play a role in mediating aspects of addiction and relapse.

POS-TUE-110

TEMPORAL EFFECTS ON HIPPOCAMPAL CELL PROLIFERATION AFTER IRRADIATION TO THE YOUNG MOUSE BRAIN

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Radiotherapy is commonly used in the treatment of brain tumors. However, there are several long-term debilitating effects after radiotherapy in children, including life-long cognitive decline. Areas in the brain where neurogenesis is present are particularly sensitive to radiotherapy and loss of these cells may contribute to cognitive deficits seen after radiotherapy. We investigated the temporal effects of irradiation on cell proliferation in the hippocampus, a region important in memory functions and one of the neurogenic areas in the brain. We irradiated mice on postnatal day (P) 14 and assessed cell proliferation and cell survival at different time points. We found a significant decrease in the number of bromodeoxyuridine-(BrdU) positive cells in mice injected at P16 and sacrificed at P22 (p=0.001). However, at P22, there was no decrease after irradiation in the number of dividing phospho-histone H3-positive cells. Furthermore, animals injected with BrdU at P23-P26 and sacrificed at P63 showed no significant difference in the number of labeled, surviving cells (BrdU-positive cells) after irradiation, but a significant difference in cell proliferation at P63 (phosho-histone H3-positive cells) (p<0.001). These results indicate that hippocampal cell proliferation initially decreases after irradiation, followed by a normalization compared to controls, eventually decreasing to a permanently low level. This may provide a window of opportunity to rescue proliferating cells and to encourage survival in order to prevent cognitive decline.

POS-TUE-111

CALORIE RESTRICTION ATTENUATES LPS-INDUCED SICKNESS BEHAVIOUR AND ALTERS IMMUNE-TO-BRAIN SIGNALLING

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This study aimed to examine the effect of calorie restriction (CR) on the development of sickness behaviour (fever, anorexia, and behavioural depression). **Methods:** Male C57BL/6J mice fed ad libitum, restricted 25% (CR25%), or restricted 50% (CR50%) in food intake for 28 days were injected on day 29 with50µg/kg of lipopolysaccharide (LPS). Changes in body temperature, locomotor activity, body weight, and food intake were determined. A separate cohort of mice were fed ad libitum or CR50% for 28 days, and hypothalamic mRNA expression of immune and metabolism compounds were determined at 0, 2, and 4 hours post-LPS. **Results:** CR50% mice did not develop fevers (p=.02 to p <.001 for the duration of the fever in controls), whereas the CR25% mice displayed a shorter-lived fever with the same peak as the controls. Both CR25% and CR50% mice showed no sign of anorexia (p <.001) and reduced cachexia (p <.001). Hypothalamic mRNA levels of NPY and CRH differed between the controls and CR50% animals at time 0; in both cases the CR50% had a significantly larger expression of NPY and CRH (p<.001 and p<.01 respectively). Of greater interest, the increases in COX-2 and mPGES observed at 2 hrs post-LPS in control mice were attenuated and delayed (p <.05); and SOCS3 expression in CR50% animals was more than 2½ times that of controls at 4 hours post-LPS (p <.001). **Conclusion:** CR results in a suppression of sickness behaviour in a dose-dependent manner, which may be due to CR causing an alteration in several compounds that mediate the release and activation of pro- and anti-inflammatory cytokines.

POS-TUE-112

EFFECTS OF AGMATINE PRE-TREATMENT ON SCOPOLAMINE-INDUCED BEHAVIOURAL DEFICITS IN THE RAT

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Purpose: Recent evidence suggests that agmatine, decarboxylated arginine, modulates learning and memory and has neuroprotective property. This study aimed to investigate whether agmatine pre-treatment could attenuate behavioural deficits induced by scopolamine, a non-selective muscarinic receptor antagonist. **Methods:** Adult male Sprague-Dawley rats were tested in the Y-maze, open field, object recognition task, Morris water maze, and elevated plus maze 30 min after the intraperitoneal injection of saline (1ml/kg) or agmatine (40 mg/kg) followed by the second injection of saline or scopolamine (0.8 mg/kg) 10 min later. **Results:** The saline-scopolamine (n=10) and agmatine-scopolamine (n=10) groups displayed reduced alternation behaviour in the Y-maze, reduced exploratory and locomotor activity in the open field, increased anxiety in the elevated plus maze, reduced time in exploring object(s) with no change in the percentage of time spent exploring the novel object in the object memory recognition task, increased swimming speed and impaired performance in the cued and place navigation, as well as probe test, in the water maze as compared to the saline-saline (n=9) and agmatine-saline (n=9) groups, respectively. Agmatine pre-treatment exacerbated scopolamine-induced deficits in the place navigation, with no marked effects on other tests. Agmatine produced a mild anxiolytic effect in saline treated animals. **Conclusion:** The present study demonstrated that scopolamine treatment altered spatial alternation behaviour, exploratory and locomotor activity, exploration of objects, spatial learning and memory, as well as anxiety level. Agmatine pre-treatment did not protect against these deficits, but even exacerbated the condition in the case of the place navigation in the water maze. A future study will investigate whether agmatine acts as a reversing agent on a pre-existent condition of cholinergic system dysfunction.

POS-TUE-113

PROTEIN KINASE A (PKA) OR CALCIUM-CALMODULIN II (CaMK II) ACTIVATION OF cAMP-RESPONSE-BINDING-ELEMENT PROTEIN (CREB) IN THE NUCLEUS ACCUMBENS REDUCES RELAPSE TO METHAMPHETAMINE-SEEKING BEHAVIOUR IN RATS

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Rationale: Methamphetamine abuse remains a large problem within society however the mechanisms that drive relapse to methamphetamine use are yet to be fully elucidated. Previous research has suggested a role for the cAMP-response-binding-element protein (CREB) in mediating drug reward processes which can be activated via two pathways, the dopamine mediated cAMP-dependent-kinase Protein Kinase A (PKA) or glutamate mediated calcium-calmodulin II (CaMK II). *Aim:* This study aimed to investigate these upstream mechanisms and the role of CREB by using a reinstatement model of drug-seeking. In particular, we examined the effect of these processes in a key reward area, the nucleus accumbens (NAc), in mediating relapse to methamphetamine-seeking. *Method:* Male Sprague Dawley rats (362 ± 5g, n=28) were surgically implanted with a jugular vein catheter and bilateral cannulae into the NAc while under isoflourane anaesthesia. One week following surgery, rats were trained to self-administer intravenous methamphetamine during daily 2 hour sessions. Methamphetamine was self-administered at a rate of 0.1mg/kg/infusion on a fixed ratio schedule for 14 days. Following behavioural extinction rats underwent 3 reinstatement test days where they were treated with an intracranial infusion of the PKA activator Sp-cAMPs (10 nmol/0.5µl/side, 20 nmol/0.5µl/side), the CaMK II inhibitor KN-93 (1 nmol/0.5µl/side, 6 nmol/0.5µl/side) or aCSF (0.5µl/side) prior to a methamphetamine priming injection (1mg/kg, i.p.). *Results:* Treatment with the PKA activator Sp-cAMPs or CaMK II inhibitor KN-93 produced dose-dependent decreases in methamphetamine-induced drug seeking behaviour when compared to infusions of aCSF. *Conclusions:* These data suggest that both PKA and CaMK II mediation of CREB are involved in relapse to methamphetamine-seeking behaviour.

POS-TUE-114

AGE DEPENDENT CHANGES IN LONG TERM POTENTIATION IN ZINC TRANSPORTER-3 KNOCKOUT MICE

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Purpose: Alzheimers disease (AD) is a common neurodegenerative disorder characterised by the cerebral accumulation of extracellular plaques, composed of the metallopeptide β Amyloid (Aβ). Transition metals such as zinc and copper play a significant role in the aggregation of the Aβ protein. In this study we have utilised electrophysiological techniques to examine long term potentiation (LTP) in ZnT3 KO and wildtype mice to assess the role of metals in normal synaptic functioning. **Methods:** LTP was measured in WT and ZnT3 KO mice at both 3 and 6 months of age and electrophysiological data obtained for further analysis. **Results:** A significant decrease in LTP was identified in 6 month old mice; average LTP in wildtype mice was 172% ± 10 (n = 5) in comparison to knockout, 106% ± 13 (n = 6). However, at 3 months, there was no change (WT: 129% ± 11 (n = 5); ZnT3KO: 124% ± 13 (n = 7)). To determine whether age-related deficit in LTP could be rescued, we supplemented slices with copper (4µm), zinc (4µm) or Clioquinol (4µm). LTP was enhanced significantly in the 6 month old KO mice with all these treatments. **Conclusion:** These data support the role of metal ions in normal synaptic function and highlight the notion that compounds that are able to restore metal ion homeostasis may be efficacious in preventing deficits in cognition.

POS-TUE-115

Cancelled

POS-TUE-116

CHARACTERISATION OF THE KYNURENINE PATHWAY IN HUMAN PRIMARY GLIOBLASTOMAS

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The kynurenine pathway (KP) is the principal route of L-tryptophan catabolism leading to the formation of the central metabolic co-factor, nicotinamide adenine dinucleotide (NAD^+), the anti-tumoral and neuroprotective agent, picolinic acid (PIC), and a number of other neuroactive metabolites. Mounting evidence indicates that the induction and over-expression of IDO-1 in various systemic tumours facilitates umour immune evasion and persistence. The intermediate α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) plays a key role in tryptophan catabolism by enzymatically driving the production of PIC. Furthermore, NAD+ is an important contributor to energy (ATP) production and plays a key role in the regulation of DNA repair, genomic stability, replication, and cell division. Most cancer cells have significantly higher energy consumption compared to non-transformed cells. **Purpose:** The KP has been fully characterised in human neuroblastoma cells. However, no literature exists characterising the KP in the most aggressive form of brain cancer, glioblastomas. **Methods:** Here we report the first characterisation of the KP in human primary glioblastoma cells (n=10) and primary cultures of human foetal astrocytes (HFA) (n=10) stimulated or not with IFN-γ (100IU/ml). **Results:** Real-time PCR revealed that human primary glioblastoma cells expressed significantly lower ACMSD but significantly higher IDO-1 compared to HFA. We also observed that glioblastoma cells produced significantly higher intracellular NAD+ concentrations compared to HFA. **Conclusion:** These findings implicates a novel mechanism involving KP dysregulation in the promotion of brain tumour cell viability and points to a number of KP products, namely ACMSD and IDO-1 as important therapeutic targets for the treatment of brain cancer.

POS-TUE-117

EFFECT OF DELAYED MESENCHYMAL STEM CELL TREATMENT FOR RAT NEONATAL HYPOXIA/ ISCHEMIA

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Purpose: Perinatal hypoxia/ischemia (H/I) is a significant contributor to neurological disabilities including motor dysfunction and cerebral palsy. No treatment is available for children affected by H/I. However, recent animal studies have shown a beneficial effect of mesenchymal stem cells (MSCs) in treating H/I. We investigated whether there was functional improvement after neonatal rat H/I following a 7-day delay in treatment with low-dose or high-dose MSCs. **Methods:** The right common carotid artery was ligated in postnatal day (PN) 7 pups followed by 1.5h of hypoxia. This induced mild/moderate brain injury. On PN14, pups received either a low-dose (85,000–120,000 cells, n=6) or a high-dose (750,000–1,000,000 cells, n=9) of bone marrow-derived rat MSCs, or diluent (n=12), injected subcutaneously. Functional outcome at PN20 was assessed using the elevated body swing, negative geotaxis, foot-fault and cylinder tests. Pups were perfused on PN21 for subsequent stereological analysis. **Results:** The cylinder test showed a higher preference of using the uninjured limb by the diluent group (37% ± 5%, mean ± SEM) compared to the high-dose MSCs group (22 % ± 6%, p=0.06, two-tailed Student's t-test). Additional pups are required to achieve statistical significance. There was no functional benefit in the low-dose MSCs group (28% ± 9%) compared to the diluent group (37% ± 5%, p=0.35). No differences were observed for the other tests. **Conclusions:** Our data confirms similar findings in mice which have shown an improvement in motor function after treatment of H/I using MSCs. Our data also suggests that delayed treatment with MSCs may improve motor function after H/I and that a high-dose of MSCs may be required for this improvement.

POS-TUE-118

CHARACTERISING THE EFFECTS OF ANTI-PSYCHOTIC MEDICATIONS ON GAMMA FREQUENCY OSCILLATIONS IN RODENTS

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Purpose: Abnormalities in gamma frequency (30 - 100 Hz) brain oscillations have been implicated in the pathophysiology of schizophrenia. In rats, treatment with the psychotomimetic compound ketamine increases gamma oscillatory power, potentially promoting this as an electrophysiological correlate of psychosis, whereas administration of antipsychotic drugs produce distinct decreases in gamma power: Haloperidol (typical antipsychotic), Clozapine (atypical) and LY379268 (preclinical $mGlur_{2/3}$ agonist with antipsychotic activity) all acutely attenuate gamma power despite their disparate pharmacology. This study aimed to characterize the duration of these antipsychotic effects. **Methods**: 6 adult male Wistar rats underwent surgical implantation of extradural electrodes to facilitate EEG recording. Following recovery, rats were connected to EEG cables and administered (s.c.) either Haloperidol (0.25 mg/kg), Clozapine (5 mg/kg), LY379628 (3 mg/kg), or vehicle (saline) and EEG was recorded for 24 hours. Treatments were >2 days apart. The EEG underwent fast fourier transformation, and average gamma power was calculated using Neuroscan software for each 30 minute interval. **Results**: All three antipsychotics produced significant ($p<0.0001$) decreases in gamma power (Haloperidol (40%), Clozapine (40%), LY379268 (60%) compared to vehicle (100%). These decreases were abrupt and were maintained for the entire recording period, with reductions of 23, 25 and 45% respectively, persisting for 24hrs post injection. **Conclusions**: The ability of antipsychotics with diverse pharmacological profiles to reduce gamma oscillatory power in rats may be related to their common clinical efficacy. Furthermore, the duration of this effect, which appears to last longer than drug presence in the brain, indicates a genomic component.

POS-TUE-119

STEM CELL TRANSPLANTATION IN TRAUMATIC SPINAL CORD INJURY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Treatment of spinal cord injury (SCI) is limited, although emerging evidence suggests that stem cell transplantation could be of some benefit. The therapeutic effects of stem cells have been assessed only in animal models of SCI, although recently the first clinical trial of oligodendrocyte progenitors derived from human embryonic stem cells has been approved. Systematic review and meta-analysis of the existing literature may provide insights into the therapeutic potential of stem cell-based therapies in animal models of SCI. **Methods:** Electronic searching was used to identify publications describing the use of stem cells in animal models of traumatic SCI where outcome was measured as a neurological score. We used DerSimonian and Laird weighted mean difference random effects meta-analysis. We assessed the impact of study design characteristics with meta-regression. **Results:** 117 publications met our pre-specified inclusion criteria. 146 experiments (2617 animals) report motor scores and 17 experiments (343 animals) report the use of sensory outcomes. Stem cells improved motor scores by 26% (95%CI 22.3-30.4). The motor score used accounts for 24% of the observed heterogeneity (adjusted r2=0.24) and the use of multiple tests provided no added benefit compared to using the BBB or BMS scales alone ($p<0.001$). A dose response relationship was observed where sensory outcomes were used ($p<0.001$) and accounted for 75% of the observed heterogeneity (adjusted r2=0.75). **Conclusions:** These data suggest stem cells improve both motor and sensory deficits in animal models of SCI. Specifically, improvements of sensory deficits are dose dependent.

POS-TUE-120

CURCUMIN AMELIORATES BEHAVIOURAL, BIOCHEMICAL, NEUROCHEMICAL AND MOLECULAR ALTERATIONS IN RESERPINE-INDUCED FIBROMYALGIA IN RATS

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Objective: The emerging concept for Fibromyalgia pathogenesis includes dysfunction of biogenic amine-mediated CNS pain control and the possible involvement of oxido-nitrosative stress-induced neurogenic inflammation. The present study was designed to investigate the effect of curcumin on the basis of its antioxidant and inflammatory potential on reserpine-induced fibromyalgia in rats. **Methods and Results** : Administration of reserpine (1 mg/kg subcutaneous daily) for three consecutive days in male wistar rats led to a significant decrease in nociceptive threshold as evident from reduced paw withdrawal threshold in Randall Sellitto and von-Frey hair test and a marked increase in immobility time. This behavioural deficit was integrated with decrease in the biogenic amines (dopamine, norepinephrine and serotonin) levels along with increased substance P concentration. Depletion in biogenic amines was coupled with increase in oxidative-nitrosative stress and inflammatory cytokines (TNF-α and IL-1β) both in serum and different brain regions (cortex and hippocampus) of the reserpinised rats. NF-kappa β and caspase-3 levels were also found to be significantly increased in both the brain regions of reserpinised rats. Curcumin (100, 200, 300 mg/kg; ip) administration ameliorated reserpine-induced nociception, depressive behaviour along with restoration of oxido-nitrosative stress, proinflammatory cytokines, biogenic amines, NF-kappa β and caspase-3 levels. **Conclusion**: The study demonstrates the effectiveness of curcumin in ameliorating the behavioral deficits associated with fibromyalgia by restoring behavioral, biochemical, neurochemical and molecular alterations against reserpine-induced fibromyalgia in rats.

POS-TUE-121

INTERACTION BETWEEN *APOE* ε4, HYPERTENSION AND BRAIN WHITE MATTER LESIONS

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Background: White matter lesions (WMLs), commonly seen as hyperintensities on MRI scans of healthy elderly individuals, are considered to be related to small vessel disease, and are often associated with a wide range of neurodegenerative and neuropsychiatric disorders. While a number of vascular risk factors for WMLs have been identified, genetic factors are also important. The *apolipoprotein E (APOE) ε4* allele is a well-established risk factor for Alzheimer's disease. Moreover, the *ε4* allele has been shown to increase the effect of cardiovascular disease on WMLs. However, the presence of the *ε4* allele alone is not a risk factor for WMLs. This study examined the effect of the *ε4* allele and its interactive effects with cardiovascular risk factors on WMLs. **Methods:** A sample of 526 Australians aged 70-90 was drawn from a larger longitudinal study, the Sydney Memory and Ageing Study. Participants provided a blood sample from which DNA was extracted. *APOE* genotypes were determined and participants were classified as either *APOE ε4* carriers or non-carriers. Participants also underwent a brain MRI scan and WMLs were quantified. The associations between *APOE ε4* and WMLs were determined using analysis of covariance, controlling for intracranial volume, age and sex. The interaction of the *APOE ε4* allele and hypertension on WMLs was also examined. **Results:** Consistent with prior reports, *APOE ε4* was not associated with WMLs before or after controlling for potential confounding factors. However, participants with hypertension and at least one *APOE ε4* allele had the highest volume of WMLs, which was predominantly driven from the protective effect of the ε4 allele in normotensive participants. The interaction was statistically significant for deep (p=0.001), periventricular (p= 0.003) and total WMLs (p=0.001). **Conclusion:** *APOE ε4* carriers are at increased risk for WMLs if they suffer from hypertension as well.

POS-TUE-122

FURTHER EVIDENCE FOR SIGNIFICANT BASAL NEUROGENESIS, INCLUDING SOME DOPAMINERGIC NEUROGENESIS, IN THE ADULT MOUSE MIDBRAIN

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Purpose: Whether or not neurogenesis and dopamine (DA) neurogenesis occurs in the adult midbrain remains controversial. Much of this controversy centres on methodologies used to detect DA neurogenesis; mostly BrdU incorporation into dividing cells. Therefore we re-examined this question using a novel transgenic approach. **Methods:** Double transgenic mice in which β-galactosidase (LacZ) expression is permanently driven in cells which express or have expressed Nestin, a marker of neural precursor cells (NPCs), in the presence of exogenously administered tamoxifen (Nes-cre ER^{T2}/GtROSA) were used. Adult mice (>8 weeks old, n=5-6/group) were administered tamoxifen (10mg) 2-3 times a week for ≤8 weeks. Brain sections were immunohistochemically processed to visualize LacZ, Neuronal Nuclei (NeuN) and Tyrosine Hydroxylase (TH). **Results:** We observed 3 areas containing high and relatively equal numbers of NPCs: (1) the subventricular zone/rostral migratory stream; (2) the hippocampal subgranular zone (SGZ); and (3) the midline region of the ventral midbrain. Further laterally in the ventral tegmental area (VTA) and substantia nigra (pars compacta and pars reticulata divisions, SNc and SNr) we observed ~1000 NeuN+/LacZ+ cells, compared with ~27000 NeuN+/LacZ+ cells in SGZ. We also observed small numbers (<50) of TH+/LacZ+ cells in the midline region and VTA. **Conclusions:** (1) The midline region of the ventral midbrain is a neurogenic niche in the adult mouse brain; (2) The basal rate of neurogenesis in and around SNc is more significant than previously thought; (3) Basal, albeit low level DA neurogenesis does occur in the ventral midbrain of adult mice.

POS-TUE-123

ALTERATION OF NEUROTROPHIC FACTOR PATHWAY GENE EXPRESSION IN THE RAT INFRALIMBIC MEDIAL PREFRONTAL CORTEX BY SUBCHRONIC RESTRAINT STRESS IS REVERSED BY FLUOXETINE

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PURPOSE: Depression is a stress-related disorder for which the neurobiological mechanisms remain poorly understood. The infralimbic medial prefrontal cortex (ILmPFC) is a cortico-limbic structure thought to modulate visceral aspects of the stress response and is thought to influence susceptibility to depression. We investigated the effects of subchronic stress and antidepressant treatment on gene expression in the ILmPFC of rats. **METHODS:** Two groups of Sprague Dawley rats (n=8/group) were maintained either in normal housing conditions or were restrained (1h/day) for 5 days. Animals were killed 24h after the final restraint and the ILmPFC dissected and processed for microarray analysis. GenomeStudio software was used to identify differentially expressed genes and qPCR carried out for confirmation of expression changes for specific genes of interest. Additionally, a fluoxetine-treated group underwent a similar protocol and qPCR analysis was carried out to determine the effects of antidepressant action on stress-induced gene changes. **RESULTS:** Our preliminary analysis showed that 24 genes were upregulated and 240 downregulated by stress, including genes related to neurotrophin factor signaling pathways. qPCR confirmed the expression change of most selected genes. Notably, fluoxetine reversed stress-induced changes in expression for the neurotrophin BDNF receptor, ntrk2, and glycogen synthase kinase-3β (gsk3β) genes, such that they were normalized back to control levels. **CONCLUSION:** These results show that sub-chronic stress induces molecular alteration in the ILmPFC and further implicates the involvement of neurotrophin factor signaling in stress-related disorders and antidepressant treatment. Future directions involve investigating the molecular changes caused by chronic stress to better understand molecular disturbances associated with the manifestation of depression and antidepressants action.

POS-TUE-124

GAS6 INCREASES MYELINATION BY OLIGODENDROCYTES AND DEFICIENCY DELAYS RECOVERY FOLLOWING CUPRIZONE-INDUCED DEMYELINATION

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Multiple sclerosis (MS) is a complex demyelinating disease of the central nervous system. The pathogenesis of MS involves many processes, including cell survival, proliferation and immunomodulation. The TAM family of receptor tyrosine kinases (Tyro3, Axl and Mertk) plays a central role in these processes. We have previously shown that in the absence of Gas6, the cognate ligand for the TAM receptors, demyelination is worsened in comparison with wild-type mice following 3 weeks of cuprizone challenge, with a corresponding increase in the loss of oligodendrocytes. We therefore hypothesised TAM receptor signalling would also influence the extent of recovery following demyelination. Gas6 knockout and wild-type mice (n=3/4 per group) were subjected to cuprizone challenge for 5 weeks. Mice were analysed at either 0, 2 or 4 weeks post-cuprizone withdrawal. Using histological and ultrastructural methods, we observed a lower level of myelination in the absence of Gas6 after 4 weeks of recovery ($p<0.05$). The delay in remyelination was accompanied by a reduction in oligodendrocyte numbers. The difference in myelination and oligodendrocyte numbers was not observed following 10 weeks of recovery, indicating that the absence of Gas6 delays, but does not ultimately prevent, remyelination. To understand the molecular mechanisms that drive these effects, we also examined the effect of Gas6 *in vitro*. We found that Gas6 significantly increased myelination in a dose-dependent manner ($p = 0.02$), suggesting that TAM receptor signalling could be directly involved in myelination by oligodendrocytes. The delay in remyelination of Gas6 knockout mice could thus result from a lack of Gas6 at a critical time during myelin production after injury.

POS-TUE-125

HIGH-RESOLUTION MRI REVEALS WIDESPREAD ABNORMAL IRON ACCUMULATION IN THE INJURED MOUSE SPINAL CORD

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Purpose: The main aim of this study was to employ high-resolution magnetic resonance imaging (MRI) to investigate the spatiotemporal development of pathological features associated with contusive spinal cord injury (SCI) in mice. **Methods:** Experimental mice were subjected to either sham surgery (n=3) or moderate contusive SCI (n=12). Non-invasive imaging of live mice as well as post-mortem fixed spinal cord specimens was performed using a 16.4T small animal MRI at sub-acute (7d; n=6) and more chronic stages (28-35d; n=4) post-injury. Routine histological techniques were used for subsequent investigation of neuropathological changes at the microscopic level. **Results:** The central core of the lesion appeared as a dark hypo-intense area on MR images at all time points investigated. Interestingly, additional loss of signal was observed spreading for at least several millimetres through the dorsal funiculi proximal and distal to site of impact, particularly in those areas undergoing Wallerian degeneration. Histological examination revealed these hypo-intense areas to be high in iron content as determined by Prussian blue staining. Quantitative image analysis confirmed the presence of distant abnormal levels of intraspinal iron at all time points ($p<0.05$). Iron deposits could also be detected via live imaging without the use of contrast-enhancing agents. Further immunohistochemical evaluation showed that intracellular iron co-localised to macrophages/microglia in the sub-acute phase following SCI but predominantly astrocytes at later stages of recovery. **Conclusion:** Widespread abnormal iron accumulation is associated with SCI in mice. High-resolution MRI can be effectively used as a diagnostic tool to monitor these neuropathological changes with time.

POS-TUE-126

ALTERED EXPRESSION OF APOPTOSIS-ASSOCIATED MESSENGER RNAS IN PREFRONTAL CORTEX FROM PATIENTS WITH SCHIZOPHRENIA

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Purpose: The observed neuropathology of schizophrenia includes a marked reduction in dendritic spines in the absence of large scale neuronal cell death consistent with apoptotic pathways having a sublethal but still deleterious role in disease development. Examination of microarray findings of the Stanley Medical Research Institute postmortem brain collection revealed altered prefrontal expression of tumour necrosis factor death receptor pathway molecules in schizophrenia, including APRIL (TNFSF13), the FAS receptor, and other changes in downstream pathway modulators and effectors. The current study sought to replicate these findings. Methods: Total RNA was isolated from dorsolateral prefrontal cortex of patients (n=72) and controls (n=71) matched on age, sex, hemisphere, and postmortem interval. Expression of target genes was measured by quantitative real-time RT-PCR. Cell culture studies were carried out using the glioblastoma cell line, U87-MG. Results: There was significant increased expression of APRIL mRNA in patients relative to controls ($U=1824.00$, $p<0.01$, $r=-0.23$) and decreased expression of BID mRNA ($t(139)=2.656$, $p<0.01$). The expression of APRIL mRNA correlated negatively with tissue pH. Treating cultures of U87-MG with APRIL did not decrease intracellular pH, nor did decreasing intracellular pH in U87-MG cells increase APRIL gene expression. Conclusion: Increased expression of APRIL was associated with decreased tissue pH, but we could not establish a direct causal relationship, suggesting a third factor may be involved. Having identified altered expression of APRIL and BID consistent with changes in apoptotic signalling, further studies are required to determine if receptors for APRIL localize at the synapse and if changes in APRIL and BID expression have effects on dendritic spine density.

POS-TUE-127

NEUROGENESIS AFTER HIPPOCAMPAL STROKE IN ADULT MICE

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Purpose: The hippocampus is vulnerable in various disease states, including stroke. We have recently discovered several populations of activatable hippocampal stem cells that represent novel therapeutic targets and hypothesise that activating these cells after stroke will elevate neurogenesis and improve function. **Methods:** Stroke was induced in adult female C57Bl/6 mice by intrahippocampal injections of the vasoconstrictor Endothelin-1. Cresyl violet staining and immunohistochemistry were used to determine lesion size as well as neuronal and astrocytic proliferative and migratory responses. Neurosphere assays were used to evaluate neurogenic potential following stroke, as well as stem cell activation in the presence of potassium or noradrenalin. **Results:** Seven days after stroke (n=10) cresyl violet staining revealed that, of the total length of the granule cell layer, 43% of the ventral and 29% of the dorsal dentate gyrus were pyknotic, representing a significant lesion compared to 2% and 3% of length respectively in the sham hemisphere. Doublecortin immunohistochemistry showed that neuron production was significantly reduced to 46% of sham levels in the ventral and 42% in the dorsal dentate gyrus, but confirmed neurogenic viability. Neurosphere production from the stroke hemisphere was reduced to 69% of sham hemisphere levels (n=11). However, proliferative cells remained activatable, as the addition of either potassium or noradrenalin increased neurosphere numbers in both sham and stroke hemispheres (potassium: sham=252%, stroke=249%, n=7; noradrenalin: sham=160%, stroke=207%, n=4). **Conclusion:** Although Endothelin-1-induced stroke results in cell death in the dentate gyrus, neurogenic potential is still preserved and the stroke hemisphere retains cells sensitive to activation by potassium and noradrenalin *in vitro*. Future work will establish whether various treatments can activate latent hippocampal stem cells, stimulate neurogenesis, and improve functional outcomes *in vivo*.

POS-TUE-128

EXACERBATION OF DEFICITS IN AMYLOID PRECURSOR PROTEIN KNOCKOUT MICE FOLLOWING TRAUMATIC BRAIN INJURY IS RESCUED WITH sAPPα

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Purpose The role of the amyloid precursor protein (APP) following traumatic brain injury (TBI) remains controversial due to the disparate functions of its breakdown products, neurotoxic Aβ and neuroprotective sAPPα. Although our previous work showed that APP-/- mice were significantly more impaired following a mild diffuse TBI than their APP+/+ counterparts, it is unclear whether this would occur with a more severe level of injury. **Methods** In this study, outcome of APP-/- mice was compared to APP+/+ mice following a moderate focal injury delivered using a controlled cortical impact device. The effect of treatment with sAPPα on injured APP-/- mice was also examined. **Results** Following injury, spatial memory was impaired in both APP+/+ and APP-/- mice on the Barnes Maze, although the injured APP-/- mice took significantly longer than APP+/+ mice to find the previously learned escape hole ($p<0.01$). These cognitive deficits were correlated with a significant increase in degenerating neurons, as detected by Flouro Jade staining, within the hippocampus and the dentate gyrus at 24 ($p<0.01$) and 72 hrs ($p<0.05$) post-injury. By 7 days post-injury the APP-/- mice had significantly less remaining neurons within these regions than APP+/+ mice ($p<0.05$). The deficits seen in the APP-/- mice were attenuated by sAPPα treatment post-injury, with the treated APP-/- animals being not significantly different from APP+/+ animals on the Barnes Maze. Furthermore, they had significantly less degenerating neurons at 24 hrs post-injury than untreated APP-/- animals ($p<0.01$), with numbers of remaining neurons within the hippocampus at 7 days post-injury no different to that in APP+/+ animals. **Conclusion** This study shows that APP remains protective with increasing levels of TBI severity, with this neuroprotective activity relating to the presence of sAPPα.

POS-TUE-129

DOES EXPRESSION OF BMP ANTAGONISTS ACCOUNT FOR MORE OLIGODENDROCYTE AND LESS SCHWANN CELL REMYELINATION IN THE CNS?

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Purpose: The role of bone morphogenetic protein (BMP) signalling following CNS demyelination is poorly understood. BMP signalling may regulate the proportion of oligodendrocyte versus Schwann cell remyelination. We expect that high levels of BMP antagonists favour oligodendrocyte remyelination, whereas low levels favour Schwann cell remyelination. **Methods:** Reverse-transcriptase PCR was used to determine mRNA expression levels of a panel of BMP antagonists in control rat spinal cord white matter (n=3) and during remyelination at 5, 14 and 28 days after induction (dai) of demyelination. Demyelination was induced either by 1% lysolecithin injection (n=9), which favours oligodendrocyte remyelination, or by 0.1% ethidium bromide (EB) injection (n=9), which favours Schwann cell remyelination. **Results:** After lysolecithin lesion induction, BMP antagonist expression increased above control at 5, 14 and 28 dai ($p \leq 0.05$). After EB lesion induction, control and 5 dai BMP antagonist expression was unchanged ($p > 0.05$), whereas at 14 and 28 dai expression increased ($p \leq 0.05$). BMP antagonist expression at 5 dai was significantly higher in lysolecithin lesions than EB ($p \leq 0.01$). **Conclusion:** These findings support our hypothesis that BMP antagonism favours oligodendrocyte remyelination. High BMP antagonist expression after lysolecithin-induced demyelination may favour oligodendrocyte remyelination by reducing BMP signalling, whereas lower expression 5 days after EB-induced demyelination may support BMP-mediated Schwann cell remyelination. Despite increased BMP antagonist levels in EB lesions after 14 days, this cannot rescue oligodendrocyte remyelination, suggesting a critical period before 14 days where remyelination type could be determined by BMP signalling. Further work should uncover the role of specific BMP antagonists during remyelination.

POS-TUE-130

EFFECTS OF HEPARIN AND ENOXAPARIN ON APP PROCESSING AND A BETA PRODUCTION IN TG2576 PRIMARY CORTICAL CELLS

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Background: Glycosaminoglycans (GAGs) have been proposed as therapeutic agents for the treatment of Alzheimer!'s disease (AD). Our previous studies have shown that GAGs can stimulate the β-site APP cleaving enzyme-1 (BACE1), which catalyses the first step in the production of the β-amyloid protein (Aβ) of Alzheimer!'s disease. Our studies have also shown that GAGs bind to β-amyloid precursor protein (APP) and can regulate its function. Recently, enoxaparin, a low molecular weight form of the GAG heparin, was reported to lower Aβ plaque deposition in APP transgenic mice. **Purpose:** In the present study, we examined whether heparin and enoxaparin can influence the APP processing and inhibit Aβ production in cortical cell cultures. **Methods:** Heparin and enoxaparin were incubated with primary cortical cells from APP (SW) Tg2576 mice, and the level of APP and proteolytic products of APP (sAPPα, C99, C83 and Aβ) were measured by western blotting. **Results:** Treatment of cortical cells with heparin or enoxaparin had no significant effect on the level of total APP. However the GAGs decreased the level of C99 and C83, and inhibited sAPPα and Aβ secretion. In contrast, both heparin and enoxaparin increased the production of a 9-kDa C-terminal APP fragment (APP-CTX). The production of APP-CTX was not inhibited by α- or β-secretase inhibitors. **Conclusion:** Our data indicate that heparin and enoxaparin stimulate an APP processing pathway that is independent of α- or β-cleavage. We propose that stimulation of this alternative pathway may be a viable therapeutic strategy for the treatment of AD.

POS-TUE-131

SUBTLE DIFFERENCES IN THE MIDBRAIN NUCLEI OF ADULT GDNF AND NEURTURIN DEFICIENT MICE

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Purpose Neurturin and GDNF are potent neurotrophic factors for midbrain neurons. Studies examining nigral dopaminergic cell populations in GDNF and Neurturin knockout mice failed to demonstrated an aberrant phenotype at birth. However, the potential for phenotypic changes in discrete subsets of dopaminergic neurons which may be differentially affected in neurodegenerative disorders such as Parkinson's Disease, requires elucidation. As dopaminergic cells expressing the calcium-binding protein, calbindin, have an increased survival potential in Parkinson's disease (Murase and McKay, 2006), we test the hypothesis for this cell phenotype to be differentially affected in mice with altered neurotrophic factor expression. **Methods** Tissue sections from 12-14 week old neurturin knockout and GDNF partial knockout mouse brains and their wildtype littermates (n=5) were sequentially labelled through the substantia nigra with tyrosine hydroxylase (TH), calbindin (CB) and calretinin (CR) antibodies. Cell numbers were quantified using 3D-fluorescence stereology. **Results** No significant difference occurred in TH+ / CB- cells numbers between the knockouts and their wildtype littermates, consistent with previous findings. However, there was a significant increase in the number of TH+ / CB+ cells and the TH- / CB+ cells in the neurturin knockouts compared with their wildtype littermates, which was not reflected in the GDNF partial knockout mice. No phenotype was seen in CR+ cells. **Conclusion** TH+ / CB+ and TH- / CB+ cell numbers increased in the absence of neurturin. Additionally, calretinin, which is also associated with calcium-binding but does not confer an increased survival potential in PD, was unaffected by Neurturin or GDNF expression. This data suggests a novel link between neurturin and the neurons expressing calbindin.

POS-TUE-132

PERINATAL NR2B ANTAGONISM: EFFECTS ON THE DEVELOPMENTAL PROFILE OF NMDA RECEPTOR BINDING AND NR2B BINDING IN THE MOUSE BRAIN

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Purpose: The NMDA receptor is critical to brain development. Perinatal NMDA receptor blockade is used as an animal model for schizophrenia aetiology. The NR2B subunit of the NMDA receptor is widely expressed during the perinatal period. The aim of this study was to determine the contribution of NR2B-containing NMDA receptors to the neurochemical effects observed in the perinatal NMDA hypofunction model. **Methods:** Male and female mice were treated with either the NR2B antagonist Ro 63-1908 (30mg/kg) or saline from postnatal day 7 to 14. Brain tissue was collected eight hours after the last treatment, at adolescence and adulthood (n=8-13 per group). Quantitative receptor autoradiography was used to determine the level of [^{3}H]Ifenprodil binding to the NR2B subunit and [^{3}H]MK-801 binding to the NMDA receptor in the prefrontal cortex and hippocampus. **Results:** There was a 15% reduction in [^{3}H] Ifenprodil binding in the treatment group eight hours after treatment ($p = 0.000$) in both brain regions. In addition, there was a slight increase in binding from eight hours after treatment to adulthood. For the [^{3}H]MK-801 binding there was an increase in binding from eight hours after treatment to both adolescence and adulthood in both brain regions. However the was no difference between the control and treatment groups at any age. **Conclusion:** NR2B antagonist treatment caused a decrease in the NR2B subunit eight hours after treatment. Although there were no long-term changes or corresponding change in total NMDA receptor binding, this model could potentially have relevance to NMDA receptor hypofunction induced effects in other neurotransmitter systems.

POS-TUE-133

SELECTIVE DISRUPTION OF SYNCHRONY BETWEEN PREFRONTAL CORTEX AND HIPPOCAMPUS IN THE MATERNAL IMMUNE ACTIVATION (MIA) MODEL OF SCHIZOPHRENIA

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Purpose. Disruption to long-range synchronised neural activity is thought to underlie several deficits in schizophrenia. We have previously reported disrupted synchrony between the medial prefrontal cortex (mPFC) and dorsal hippocampus (HPC) in the MIA model in rats. The current study sought to determine whether this was specific to communication between these two regions. **Methods**. We examined synchronised neural activity between the mPFC and the dorsal and ventral HPC (dHPC & vHPC) in MIA (n=10, from 8 litters) and control (n=9, from 8 litters) animals. The MIA model of schizophrenia is based on epidemiological evidence of increased risk of schizophrenia in adulthood following prenatal exposure to infection and is induced through a single injection of the synthetic immune system activator polyriboinosinic-polyribocytidylic acid (Poly I:C), in pregnant rat dams. EEG was recorded simultaneously from dorsal and ventral HPC and mPFC in freely moving MIA and control offspring foraging in an open field environment. **Results**. Although the MIA intervention produced significant reductions in mPFC-dHPC EEG coherence in multiple frequency bands (all $p<0.05$), no reduction in mPFC-vHPC coherence was found for any band. There was a significant group by region interaction in delta, theta, and beta frequency bands (all $p<0.05$), highlighting the specificity of the deficit. **Conclusions**. MIA in rats produces a fundamental disruption in long-range neuronal synchrony between the dHPC and mPFC in adult offspring. Our data show that this is specific to this hippocampal region. This finding provides important direction for further research as it will allow for comparative investigation of functional and dysfunctional long-range communication. It also refines the search for aberrant pathways and highlights a potential target for therapeutic intervention.

POS-TUE-134

DO LEVELS OF PROLIFERATION-REGULATING FACTORS CHANGE WITH AGE IN HUMAN ADULT NEUROGENIC REGIONS?

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Neurogenesis, the birth of new neurons from precursors, continues throughout adulthood in the human subventricular zone and hippocampus. It is not known how levels of factors thought to regulate the proliferation of precursors change with age in human adult neurogenic areas. **Purpose and Methods:** The current project employed ELISAs to investigate changes in levels of putative proliferation-regulating factors in the healthy human subventricular zone and hippocampus throughout the adult lifespan (18 to 104 years). **Results:** Human hippocampal levels of the anti-proliferative transforming growth factor-β1 strongly increased with age ($n=10$, $p=0.005$), whilst human hippocampal and subventricular zone levels of the pro-proliferative basic fibroblast growth factor, epidermal growth factor, glial-derived neurotrophic factor, heparin-binding epidermal growth factor, insulin-like growth factor-1, transforming growth factor-α and the anti-proliferative interleukin-1β and interleukin-6 did not change significantly with age. Levels of brain-derived neurotrophic factor, basic fibroblast growth factor and interleukin-1β were significantly higher in the hippocampus than in the subventricular zone and levels of glial-derived neurotrophic factor and transforming growth factor-α were significantly higher in the subventricular zone ($p<0.005$), suggesting that factors with predominant influences on neurogenesis differ between the two human adult neurogenic areas. **Conclusion:** These findings suggest regulation of the adult neurogenic environment in the human brain may differ over time from that in other species.

POS-TUE-135

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

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Chronic stress has been implicated as a causal factor in depression, and is associated with neuroendocrine dysfunction and impaired hippocampal neurogenesis. Depressed patients exhibit reduced levels of the neurosteroid allopregnanolone (ALLO). ALLO is involved in regulating neuroendocrine activity. We propose chronic stress decreases ALLO production leading to over-activation of the neuroendocrine system. **Purpose**: This study investigated whether exogenous ALLO could prevent development of depressive-like behaviours in chronically stressed rats, and maintain hippocampal neurogenesis. **Methods**: Chronic stress was induced by social isolation (SI) of male Wistar rats for 6 weeks. A subcutaneous pellet containing ALLO (1mg/day) or placebo was implanted at the time of isolation. Depressive-like behaviour was assessed using the novelty-suppressed feeding (NSF) test and forced-swim (FS) test. Animals were injected with IdU and CldU to assess progenitor cell proliferation and survival, respectively. Cell counts were performed to estimate total hippocampal CldU+ and IdU+ cells. Comparisons were against group-housed controls (GH), placebo-treated SI animals (SI-placebo) and SI animals treated with the antidepressant, fluoxetine (SI-FLX) (n=7/group). **Results**: After six weeks of isolation the SI-placebo group took significantly longer than GH ($p<0.001$), SI-ALLO ($p=0.03$) and SI-FLX ($p=0.008$) treatment groups to feed in the NSF test, and spent more time immobile in FS test than GH animals ($p=0.02$).No difference was seen between behaviour of SI-ALLO and either GH or SI-FLX treated animals ($p>0.05$). ALLO treatment during SI also maintained the proliferation and survival of hippocampal progenitor cells when compared to GH animals. **Conclusions**: Administration of exogenous ALLO during SI-induced chronic stress prevents the onset of depressive-like behaviour and maintains hippocampal neurogenesis. ALLO may provide a novel therapeutic target for the treatment of depression.

POS-TUE-136

ADVANCED PATERNAL AGE AND COPY NUMBER VARIATION IN C57BL/6J MICE

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Background: Advanced paternal age (APA) is associated with neurodevelopmental disorders including autism, epilepsy and schizophrenia. Increases in genomic copy number variation (CNV) in the male germ line may be transmitted to the next generation. The aim of this study was to investigate the impact of APA on CNV load in offspring using C57BL/6J mice and array comparative genomic hybridization (aCGH). **Methods:** 4 month old ('young') and 12 months old ('old') sires were mated with 4 month old dams to create young paternal age (YPA) and APA offspring (n=6 in each group of offspring). Tail-tip DNA from parents and offspring were hybridized against a reference sample to 44k custom arrays (designed by S. Chong) and 244k off-the-shelf arrays (both supplied by Agilent). CNVs detected with CGH Analytics software (Agilent) were separated into '*de novo*' (present in offspring only) and 'inherited' (present in offspring and at least 1 parent). **Results:** Two separate *de novo* CNVs were evident, 1 in YPA and 1 in APA offspring. The latter was located within *Auts2*. Five distinct inherited CNVs were observed and these were associated with specific families. **Conclusions:** There was no increased *de novo* CNV load in APA offspring. The *de novo* CNV within *Auts2* in the APA offspring is of interest to our research as abnormalities in this gene have been associated with autism, epilepsy and mental retardation. Additionally, the results reveal the need to consider the occurrence of CNVs in studies using C57BL/6J mice.

POS-TUE-137

REGULATION OF THE CELL ADHESION MOLECULE, L1CAM BY THE Aβ PEPTIDE OF ALZHEIMERS DISEASE

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Alzheimer's disease (AD) is a progressive neurological disorder underpinned by the toxic action of the amyloid-β peptide (Aβ). A prominent histopathological feature of AD is the presence of dystrophic neurites surrounding amyloid plaques. There is evidence suggesting that the Aβ peptide disrupts neuronal adhesion in vitro, leading to neuritic dysfunction, a possible correlate of the significant synaptic dysfunction that contributes to the cognitive decline seen in AD patients. In this study we asked whether L1CAM, an adhesion molecule with crucially important roles in cell migration and axonal fasciculation during development is misregulated in response to the toxic actions of Aβ peptide. Using a polyclonal antibody, we measured L1CAM expression in cultured cortical neurons exposed to $A\beta_{1-42}$. Significant increases in L1CAM immunoreactivity were seen in Aβ-treated cell soma and dendrites at 10, 11 and 12 DIV ($p < 0.05$, n=48 cells), when compared to cells treated with vehicle (DMSO) only. We next asked if L1CAM expression was misregulated in the brain of a transgenic mouse model of AD. Surprisingly, L1CAM expression was significantly increased in white matter of 3-5 month old Tg2576 mice, well before the onset of any AD related pathology. These results were confirmed by western immunoblot analysis which showed a significant increase in full-length L1CAM in the brain of Tg2576 mice, when compared to their wild-type littermates. These findings confirm that L1CAM expression is upregulated in response to Aβ peptide in vivo. One possible mechanism for such an action may involve a compensatory upregulation of L1CAM at sites of early synaptic dysfunction in response to Aβ toxicity.

POS-TUE-138

NOVEL METAL LIGANDS ARE NEUROPROTECTIVE AND RESTORE MOTOR DEFICITS IN ANIMAL MODELS OF PARKINSON'S DISEASE

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Background: Parkinson's disease (PD), is the second most prevalent neurological disorder in Australia. While the cause of PD remains poorly understood, we have some clues as to why the damage, once initiated, progresses inexorable. Damage to neurons of the Substantia nigra (SN) may be related to pathological interactions between α-synuclein, dopamine and redox active metals (iron). The moderate-affinity metal ligand, Clioquinol (CQ) has shown to protect SN neurons, presumably by preventing the participation of iron in unregulated oxidative processes. **Purpose:** To prevent or slow SN degeneration by developing novel neuroprotective compounds possessing CQ-like metal binding affinity. **Methods:** Initial screening of a novel chemical library bearing the CQ metal ligand motif ranked the ability of test compounds to silence or reduce redox activity. Adapting 6-OHDA and MPTP animal models to reflect a "treatment" rather than a "prevention" paradigm drug was administered only after the cell death cascade was initiated and toxin has been cleared from the body. The neuroprotective property of test compounds was assessed by stereological live-cell counting; behaviour modifying ability by amphetamine induced rotations (6-OHDA model) and by the fastest turn and completion times in the pole test (MPTP). **Results:** Lead compound PBT434 preserved over 50% more SN neurons compared with untreated MPTP (n=10) or 6-OHDA (n= 6) lesioned (n=10) counterparts ($p<0.001$). PBT434 reduced amphetamine induced rotations by 60% ($p<0.05$) and normalised motor behaviour in the Pole test (n=12). PBT434 also abolished the increase in a global brain iron and α-synuclein levels seen after the toxins were administered (n=5). **Conclusion:** Novel compounds aimed at preventing injury dependent rise in nigral iron provide strong neuroprotection and maintain motor function. A new generation of disease modifying therapies will include such drugs designed to target key pathways in the degeneration of SN cells.

POS-TUE-139

A NEW RAT MODEL FOR THE PREDISPOSITION TOWARDS EPILEPSY, ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) AND AUTISM SPECTRUM DISORDERS (ASD)

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Purpose: The degree of clinical overlap in patients with epilepsy, ADHD and ASD is highly suggestive of a common predisposing mechanism. Accordingly, a rat strain selectively bred to be seizure-prone (FAST), versus seizure-resistant (SLOW), naturally developed comorbid behavioral patterns reminiscent of ADHD/ASD in humans, including relative hyperactivity, impulsivity, social/learning deficits and a substantial neurodevelopmental delay. Thus, FAST rats appear to be a natural model system in which to study mechanisms underlying vulnerability to these interrelated disorders. Interestingly, relative to SLOW rats, FAST rats show reduced levels of circulating free fatty acids (FFA) despite maintenance on an identical diet. If this deficit were maintained throughout pregnancy/lactation it could substantially impact offspring neurodevelopment given the broad functional mandate of fatty acids (FA) in neurogenesis and myelination. **Methods**: FFA levels were compared in plasma extracted from FAST (N=40) and SLOW (N=40) female rats under control conditions and after 1, 2 or 3 weeks of pregnancy. Comparative analysis of individual FA levels across lipid fractions was also conducted in order to determine whether this deficit reflected specific FA deficiencies or reduced FA levels more generally. **Results:** Significantly reduced FFA levels were confirmed in FAST rats at each stage of pregnancy. This deficiency, however, was specific to certain long chain FAs, suggesting Fast rats may be unable to efficiently elongate essential FA precursors into their long chain derivatives. **Conclusion:** These data indicate that native differences in lipid handling have evolved in the seizure-prone FAST versus seizure-resistant SLOW rats. Similar aberrations in lipid handling have been reported in patients with ADHD/ASD.

POS-TUE-140

NDFIP1 PROTECTS NEURON FROM DEATH FOLLOWING BRAIN INJURY

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Introduction: The removal of harmful proteins is an important mechanism for ensuring neuron survival following injury. Ndfip1 is an important player in the ubiquitination and degradation of target proteins through the Nedd4 ligase pathway. Therefore genetic manipulation of Ndfip1 expression would be expected to modify the animal response to brain injury. **Purpose:** To examine the effect of brain injury on animals lacking a copy of the Ndfip1 gene. **Methods:** Ndfip1 heterozygous mice and wild-type littermates were used. The mice received a closed head injury using an electric weight-drop device and euthanized 24 hours later. The lesion volume was quantified in serial coronal sections, using 2,3,5-triphenyltetrazolium chloride (TTC) staining. **Results:** There is significant increased of cortical lesion volume ($p<0.05$) in Ndfip1-heterozygous mice (n=7) compared to their wild-type littermates (n=7). **Conclusion:** The reduction of Ndfip1 in the brains of heterozygous mice leads to increased susceptibility to injury, resulting in larger injury areas. Ndfip1 is an important player for neuron protection following brain injury.

POS-TUE-141

INTERLEUKIN-17 KNOCKOUT MICE HAVE IMPROVED LOCOMOTOR RECOVERY, SMALLER LESION SIZE, AND REDUCED LEUKOCYTE RECRUITMENT FOLLOWING SPINAL CORD CONTUSION INJURY

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Following the initial impact, spinal cord injury (SCI) triggers a number of inflammatory responses which can exacerbate tissue damage in the cord and impair functional recovery. The involvement of several pro-inflammatory cytokines in the secondary degenerative mechanisms of SCI has been well established, although the role of interleukin-17 (IL-17) remains unclear. In the present study, we used IL-17 knockout (KO) and C57BL/6J wildtype (WT) mice to investigate the effects of IL-17 deficiency on locomotor recovery and neuroinflammation following a contusion injury. 16 IL-17 KO and 14 WT mice underwent a contusion injury using a modified New York University Impactor. Four control mice had sham operations. Hindlimb locomotor function was assessed in an open field using the BMS scoring system, and lesion size, glial cell activation and inflammatory response were analysed using immunohistochemistry following spinal cord contusion injury. Our results show that compared to WT mice, IL-17 KO mice had a significantly smaller lesion size, which corresponded with a significantly better locomotor functional recovery following SCI. There were reductions in the number of neutrophils, B cells and dendritic cells in the IL-17 KO mice but no difference between IL-17 KO and WT mice in the presence of activated microglia and reactive astrocytes in the injured spinal cord 6 weeks after injury. These findings suggest that IL-17 is a mediator of secondary degeneration following SCI, which hinders functional recovery, though its actions do not affect glial activation in the injured cord.

POS-TUE-142

GENETIC INFLUENCE OF MAPT ON THE PATHOLOGY OF PARKINSON'S DISEASE

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Background: Recent genome-wide association studies show that *SNCA* (α-synuclein) and *MAPT* are risk genes for sporadic Parkinson's disease (PD), complementing previous association studies showing that the *MAPT* H1 haplotype is more frequent in PD. **Objective:** To assess whether the *MAPT* H1 haplotype influences the levels of tau protein and its isoforms and relates to the severity of pathology in PD. **Methods:** Cerebellar brain samples (a region not affected by PD) from 116 PD cases were obtained through the Australian Brain Bank Network following study approval by University of NSW Human Research Ethics Advisory Panel. Genomic DNA was extracted and *MAPT* H1 haplotyping performed. Severity scores for diagnostic neuropathologies were extracted from neuropathological reports (mild, moderate or severe substantia nigra neuronal loss; Braak α-synuclein PD stages 4, 5 or 6; Braak tau neuritic stages 0-6). Twenty-seven cases with similar demographics and different *MAPT* H1 genotypes were selected for quantitation of total tau and its isoforms. The remaining brain samples were then homogenized and sonicated. Semiquantitative Western blotting was performed using specific tau antibodies. Associations between *MAPT* haplotype and pathological variables were determined using SPSS covarying for age and disease duration. **Results:** 4-repeat tau protein levels, but not total tau, were increased in H1 haplotype carriers without changing the severity of tau neuropathologies. *MAPT* haplotype did not influence the severity of nigral cell loss or Braak PD stage. **Conclusion:** This data shows that the *MAPT* H1 haplotype influences the ratio of tau isoforms in the brain (more 4-repeat tau in H1 carriers). This change in tau isoform expression did not influence the deposition of tau protein or the pathological progression of PD.

POS-TUE-143

HUNTINGTON'S DISEASE SHEEP MODEL INVESTIGATED USING NEXT GENERATION TRANSCRIPTOME SEQUENCING TO IDENTIFY EARLY CHANGES IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a late-onset neurodegenerative disorder caused by a single genetic mutation in the IT15 gene. Mostly rodent models are being used to investigate the disorder. In 2005 a transgenic ovine model of HD was made. The transgene consists of the full length human cDNA driven by the minimal human promoter. Recently, next generation sequencing (NGS) was performed on two pools of RNA extracted from the cortex of 2 transgenic and 2 control 18 month old sheep. **Purpose**: Identify from the animals' transcriptome profiles, early differential gene expression in the transgenic HD sheep brain, with emphasis on early markers of disease and potential biomarkers. **Methods:** NGS was performed at GeneWorks, Adelaide, using an Illumina platform to generate paired end reads of 65bp. Data was analysed at the University of Auckland Bioinformatics Institute using Tophat and Cufflinks software. **Results:** 24,419,789 control and 28,214,424 transgenic paired end reads were initially mapped to a bovine genome. Genes of interest were selected from quantified transcripts which indicated differential expression in the transgenic sheep. These are currently being examined by quantitative PCR in multiple transgenic and control animals (1-18 months of age) to assess relevance to HD. Once an ovine genome is completed, the NGS data will also be aligned to this to gain more precise and encompassing expression information. **Conclusion:** NGS has provided a unique opportunity for a transcriptome-wide, quantitative analysis of gene expression in a symptom-free animal model of HD. Changes identified represent some of the earliest events in the molecular process of HD. Altered genes thus have potential as biomarkers, particularly those that are also expressed in easily accessible peripheral tissues such as blood.

POS-TUE-144

ANGIOGENESIS AFTER STROKE IS DEPENDENT ON LESION SIZE AND ASSOCIATED WITH EXTENSIVE NEURONAL LOSS

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The severity of ischemic stroke is variable in patients and may affect subsequent pathophysiological responses. Cerebral ischemia induces angiogenesis within and around infarcted tissue however the effect of damage on angiogenesis is yet to be determined. **Purpose**: To investigate the effect of stroke lesion on the extent of angiogenesis in a rat model of stroke with reperfusion. **Methods**: The middle cerebral artery was constricted by endothelin-1 (ET-1) in conscious rats (n=9). Neurological and histological outcome was assessed by neurological deficit score and MCID image analysis. Blood vessels were detected *in situ* using Von Willebrand Factor and point counted using Metamorph imaging software 14 days post-stroke. **Results**: Stroke induced by ET-1 vasoconstriction resulted in significant neurological deficits detected between 1 and 7 days but not after this time (P<0.001). Histopathology revealed varying degrees of damage in the ipsilateral cortex and striatum with volumes ranging from 3-40 mm^3. Angiogenesis was assessed in the infarcted cortex, striatum and surrounding border zone and compared with the corresponding mirror image on the contralateral side. Blood vessel counts revealed larger stroke volumes correlated significantly with higher levels of angiogenesis in the core cortical infarct (r=0.64, P<0.01), core striatal infarct (r=0.43, P<0.05) and surrounding border zone (r=0.69, P<0.01). Immunohistochemical localisation of neurons (NeuN) and blood vessels (vWF) post-stroke showed concentrated angiogenesis in brain regions with greatest neuronal loss. **Conclusion**: Angiogenesis represents an attempt at brain repair and is associated with improved functional outcomes 14 days post-stroke. Stroke severity affects subsequent levels of angiogenesis and presents an important platform for promoting recovery.

POS-TUE-145

INTERACTION OF THE RECEPTOR-ASSOCIATED PROTEIN (RAP) WITH APP AND ABETA

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Background: Alzheimer's disease (AD) is characterized by an accumulation of amyloid peptide (Aβ) in the brain. Recently, the 39-kDa receptor-associated protein (RAP) has been implicated in Aβ pathology in a transgenic mouse model of AD. We have shown that RAP binds strongly to Aβ, leading to an inhibition of its aggregation and neurotoxic effects. Furthermore, our studies show that RAP is decreased in the AD brain. **Purpose:** The study aimed to examine the region of RAP which binds to Aβ and the effect of RAP overexpression on APP metabolism and Aβ production. **Methods:** The self-association of Aβ was measured using a plate assay. To examine the effect of RAP on APP metabolism, APP-CHO cells were transfected with RAP pcDNA3.1 plasmids and the level of APP and its proteolytic products (sAPPα, C83, C99 and Aβ) were measured by western blotting. **Results:** RAP fragments containing a region between domains 2 and 3 were found to block Aβ self-association. Fragments lacking this region did not have any effect. We found that overexpression of RAP had no significant effect on the level of total APP. However, the level of C99 and Aβ was decreased in RAP-transfected cells. In contrast, the production of sAPPα and C83 was significantly increased. **Conclusion:** We conclude that Aβ binds to RAP through a loop region containing 10 amino-acid residues located between domains 2 and 3. Furthermore, overexpression of RAP influences the APP processing by decreasing β-cleavage and increasing α-cleavage of APP. The data suggest that RAP is a protective factor for Alzheimer's disease.

POS-TUE-146

NEUROPROTECTION BY SELECTIVE NITRIC OXIDE SYNTHASE INHIBITION AT 48 HOURS AFTER PERINATAL HYPOXIA-ISCHEMIA

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Purpose: A major pathway of neuronal injury following hypoxia-ischemia and reperfusion is the production of excessive nitric oxide and reactive oxygen species. The developing brain is particularly vulnerable as endogenous antioxidant systems are poorly developed. Previous studies have reported neuroprotection with 2-iminobiotin (2IB), a selective inhibitor of neuronal and inducible nitric oxide synthase. The present study aimed to determine the most effective therapeutic dose of 2IB in a piglet model of perinatal hypoxia-ischemia. **Methods:** Newborn piglets (N=38) were subjected to 30min of hypoxia with a final 10min period of hypotension. At the end of insult, animals were randomly treated with either vehicle (N=12), or 2IB 0.1mg/kg (N=8), 0.2mg/kg (N=10), or 1.0mg/kg (N=8), with repeat doses given intravenously every 4h until 20h post-insult. Brain activity was assessed for 30min using EEG at each dose and at 24 and 48h post-insult. Animals were euthanased at 48h post-insult and brains processed for measurement of caspase-3 activity, amount of tyrosine nitration, and assessment of histopathology using haematoxylin and eosin staining. **Results:** 2IB significantly reduced caspase-3 activity by 65% in the parietal cortex and basal ganglia (0.2mg/kg dosage) compared to vehicle treated animals ($p<0.05$). A similar trend was observed in the temporal cortex ($p=0.10$). **Conclusions:** Our preliminary analysis shows that 2IB protects the brain from hypoxic-ischemic injury. Further evaluation of the most effective dose is currently underway using measures of tyrosine nitration, histopathology scores and EEG background pattern and seizure activity.

POS-TUE-147

PRONEURAL TRANSCRIPTION FACTORS DLX2 AND PAX6 ARE ALTERED IN ADULT SVZ NEURAL PRECURSOR CELLS FOLLOWING STRIATAL CELL LOSS

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Compensatory replacement of neurons by endogenous subventricular zone (SVZ)-derived neural precursor cells has been demonstrated in the adult brain following striatal cell loss. Such cell replacement is associated with increased SVZ cell proliferation and neuroblast expansion in the rostral migratory stream (RMS). SVZ-derived neural precursor cells co-express multiple transcription factors involved in lineage restriction and cell fate determination. We propose that compensatory neurogenesis will alter the temporal expression of transcription factors in discrete populations of SVZ-derived neural precursor cells. **Methods:** We examined the expression of Mash1, Dlx2, Pax6 and Olig2 in SVZ-derived neural precursor cell populations using immunohistochemistry across a range of times following quinolinic acid (QA) induced striatal cell death (n=3-5). **Results:** We have identified a heterogeneous population of SVZ-derived neural precursor cells that respond independently to striatal cell loss. In both the anterior SVZ (aSVZ) and RMS we observed an increase in a sub-population of Dlx2+ transit amplifying precursor (TAP) cells and neuroblasts following QA lesioning when compared to controls ($p<0.05$). Subsequently, the number of Pax6+ TAPs and neuroblasts in the QA lesioned aSVZ and RMS was also increased ($p<0.05$). Olig2 expression was not however altered in response to QA-induced cell loss. **Conclusion:** Our results suggest Dlx2 and Pax6 may play a prominent role in directing neural precursor cell proliferation and neuroblast generation following striatal cell loss. **Purpose:** Selective alteration of specific transcription factors in the SVZ and RMS in response to cell loss may predetermine the subsequent generation of specific neuronal subclasses for endogenous replacement.

POS-TUE-148

DELETION OF THE INTERFERON α RECEPTOR1 (IFNAR1) SUBUNIT OF THE TYPE I INTERFERON RECEPTOR CONFERS PROTECTION TO NEURONAL TISSUE FOLLOWING TRAUMATIC BRAIN INJURY

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Introduction: Traumatic Brain Injury (TBI) is the leading cause of death and disability for children and young adults. TBI leads to the development of secondary neuronal damage, such as neuroinflammation and consequent cell death. Key players in the neuroinflammatory cascade are the Type-I Interferons (IFNs), which signal through the Interferon α Receptor (IFNAR). IFNAR, comprising two subunits, IFNAR1 and IFNAR2, signals through the JAK-STAT pathway, leading to increased pro-inflammatory gene transcription. Interferon signalling is a promising target in the study of neuroinflammation after TBI. **Purpose:** To investigate the contribution of Interferon signalling in the progression of neural injury seen in TBI. **Methods:**TBI was induced using a computer-controlled impactor with a 2mm diameter tip delivering an injury above the right parietal cortex of 8-week-old male C57BL/6J wild type (WT) and IFNAR1-/- mice. Brains were excised 24 hours after TBI, used for infarct analysis and immunohistochemistry, and at 2, 4 and 24 hours after TBI, were used for qRT-PCR. In vivo MRI analysis was performed both 2 and 24 hours after TBI as an additional indication of the development of oedema and infarct. **Results:** IFNAR1-/- mice had an infarct volume that was 30% of the WT mice 24 hours post-TBI ($p\leq0.05$, n=3). Furthermore, mRNA levels for the pro-inflammatory genes IFNα, IFNβ, IL-1β and TNFα decreased in the IFNAR1-/- by 5-60 fold compared to WT. In addition, there were less Mac-1 positive cells in the IFNAR1-/- 24 hours after TBI, highlighting a lower level of invading cells compared to WT. **Discussion:** These results indicate that interferon signalling plays a deleterious role in the development of the neural injury seen in TBI. The current data suggests that the IFNAR receptor complex is an attractive target in future therapeutics involved in the treatment of TBI and other acute neural injuries.

POS-TUE-149

VARIABLE CORTICAL INTERNEURONAL LOSS IN HUNTINGTON'S DISEASE HUMAN BRAIN CORRELATES WITH SYMPTOM PROFILES

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Purpose: Our recent studies have shown that the variable symptomatology in Huntington's disease (HD) correlates with variable pattern of pyramidal cell loss in the cerebral cortex (*Thu et al., Brain: 2010*). We are now extending these studies to the cortical interneurons to determine whether the variation in HD symptom profiles also correlates with the pattern of interneuronal cell loss. **Methods:** Unbiased stereological counting was used to quantify parvalbumin, calbindin, and calretinin interneurons in the motor and cingulate cortices (13 HD and 14 control cases). The HD cases were categorized into 3 symptom groups (mood, motor, or mixed). **Results:** A variable heterogeneous pattern of interneuronal loss was observed across the two cortical regions in different HD cases. The *HD mood* cases showed a significant cell loss in all three interneuronal populations in the cingulate cortex (80% loss of parvalbumin$^+$, 71% loss of calbindin$^+$, 60% loss of calretinin$^+$ cells), but no significant cell loss in the motor cortex. By contrast, the *HD motor* cases showed a selective loss of only calbindin$^+$ interneurons in the motor cortex (57% loss), but no cell loss in the cingulate cortex. The *HD mixed* cases showed a variable interneuronal loss in the two cortical regions. **Conclusion:** There is heterogeneity in the pattern of interneuronal loss in the cerebral cortex suggesting variable pathways of pathogenesis in HD which correlates with the symptom profiles.

POS-TUE-150

STEM CELL THERAPY IN STROKE – CELLULAR MECHANISMS OF ACTION

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Stroke is the leading cause of disability in Australia and New Zealand. Repair of the brain following stroke is one of the major challenges to neuroscience. The first Phase I human trial using a human foetal neural stem cell has started to treat disability post-stroke. We have published extensively on a human adult stem cell type – dental pulp stem cell (DPSC). There is poor understanding as to the underlying cellular and molecular mechanisms of action of stem cell therapy. **Purpose:** To investigate the behaviour of DPSC transplanted into the brain 24 hours post-stroke. **Method:** Reversible middle cerebral artery occlusion (MCAo) model of focal cerebral ischaemia in rodent was used. 24-hours post-stroke $6x10^5$ human DPSC were injected peri-infarct. Human DPSC were distinguished from rodent tissue by staining for green fluorescent protein (GFP; DPSC transduced to express GFP) or human mitochondrial antigen (hMitAg). This study is ongoing to investigate the neuro-behavioural outcome of DPSC treatment compared to control (to date; n=54 animals). **Results:** Preliminary neuro-behavioural results of improvement were presented in ASN 2010. Furthermore, we have found human DPSC survived long-term and predominantly resided in the peri-infarct region. In three animals that demonstrated marked neuro-behavioural improvement the whole brain was sectioned at 10 µm in the coronal plane and stained for hMitAg. Counting every second section we found 9,458 to 22,394 DPSC present in the stroke brains – 2.3% average from the original number of transplanted DPSC. We have data regarding the long-range migration, neural and angiogenic differentiation potential of DPSC in the stroke brain. **Conclusion:** Evidence from this study indicates a wide variety of cellular behaviours post-stroke transplantation of human adult stem cells.

POS-TUE-151

FRONTAL LOBE WHITE MATTER INJURY CORRELATES OF REDUCED VISUAL ACUITY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Purpose: Patients with multiple sclerosis (MS) commonly present with reduced visual acuity resulting from optic nerve inflammation. Magnetic resonance imaging (MRI) techniques have enabled the quantification of extant white matter injury in the primary visual pathway. However, we have found that the degree of visual acuity loss does not correlate with injury to the visual pathway. Therefore, we hypothesised that loss of visual acuity might be attributable to injury to higher level visual processing regions involved in directing eye movements and character recognition. We aimed to use voxelwise analytical methods to assess white matter injuries that correlate with loss of visual acuity. Methods: Whole brain diffusion tensor imaging (DTI) data from 23 patients with MS were acquired using a 3T MRI system. All patients had previous unilateral optic nerve inflammation with incomplete recovery of visual acuity (<6/7.5). Diffusion tensor imaging data from each patient were co-registered to a stereotaxic template using a DTI registration algorithm and voxelwise statistics were performed on the mean diffusivity (MD) and fractional anisotropy (FA) maps. A general linear model was used to identify voxels in which MD or FA significantly correlated with visual acuity. A conservative p-value ($p<0.005$) and cluster threshold (>100 voxels) was used to correct for multiple comparisons. Results: Significant group differences in FA were observed between patients and controls in frontal and temporal lobe white matter and in MD in frontal white matter including regions associated with visual attention. Conclusion: Permanent loss of visual acuity following injury to the visual pathway may be mediated by injury to higher order visual processing regions in the frontal and temporal lobes.

POS-TUE-152

EXPRESSION OF C5A AND ITS RECEPTORS IN THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Purpose: The complement system has recently been implicated in pathogenesis of Huntington's disease (HD). Previous studies have shown mRNA up-regulation of several complement components in HD patients compared to normal individuals. The current study aimed to determine the spatial and temporal expression of the complement molecule C5/C5a and its two receptors CD88 and C5L2, in the R6/1 mouse model of HD. **Methods:** CBB6 R6/1 mice and their wild-type (WT) littermates were examined at 4 different ages of HD progression: 6 weeks for pre-symptomatic; 12 weeks for onset of cognitive deficit; 20 weeks for onset of motor dysfunction and 28 weeks as HD end stage. At each age, mRNA expression levels and mRNA cellular localisation of C5, CD88 and C5L2 were investigated by qPCR and in-situ hybridisation (n=3/age). Protein levels of CD88 and C5L2 were also determined using immuno-blotting and localization within striatum and cortex was determined by immuno-histochemistry (n=3/age). **Results:** CD88 mRNA and protein levels were increased at 20 and 28 weeks in R6/1 mice compared to WT littermates ($P<0.05$). CD88 mRNA and protein were localized on proliferating microglia in R6/1 mice. By contrast, in WT littermates CD88 was found only on striatal and cortical neurons at all ages. C5L2 protein expression showed a similar profile to that of CD88 in both WT and R6/1 mice, except C5L2 was present on proliferating astrocytes during disease progression in R6/1 mice. **Conclusion:** These results indicate that the expression of C5a and its receptors is increased in R6/1 mice. CD88 and C5L2 have different glial expression patterns indicating potentially differential roles for these receptors in the progression of HD.

POS-TUE-153

INHIBITION OF SRY IN THE SUBSTANTIA NIGRA REDUCES STRIATAL DOPAMINE LEVELS AND IMPAIRS MOTOR FUNCTION IN MALE RATS

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Recent studies suggest that sex-specific genes, such as SRY, may influence gender differences in brain function and behaviour. SRY (Sex-determining Region on the Y chromosome) is a male sex-determination transcription factor that directs embryonic gonads to develop as testes. SRY mRNA and protein is expressed in various brain regions including the substantia nigra pars compacta (SNc), ventral tegmental area, and locus coeruleus, which are all abundant in catecholamines. In the rodent and human SNc, SRY co-localizes with tyrosine hydroxylase (TH) positive neurons. Furthermore, inhibition of nigral SRY reduces TH expression in the SNc and induces motor impairment in rats (Dewing et al., 2006). The aim of this study was to determine whether SRY regulates motor function by regulating the production of nigrostriatal dopamine in males. To test this aim, we assessed the effect of SRY inhibition on motor function and striatal dopamine levels, by chronic infusion of SRY antisense (or sense control) oligonucleotide into the SNc in male rats. Motor function was assessed, before (day 0) and after the treatment (day 14), by cylinder and rotarod tests. Rats were culled on day 15 and striata were isolated for dopamine and DOPAC measurements. The cylinder test revealed that chronic injection of SRY antisense reduced contralateral limb usage on day 14, compared to day 0 (-21%, P>0.05) and sense-treated control on day 14 (-19%, P>0.05). Similarly, SRY antisense impaired performance on the rotarod test on day 14, compared to day 0 (-15%, P>0.05) and sense-treated control on day 14 (-14%, P>0.05). The impairment in motor function following SRY antisense injections was associated with a significant reduction in striatal dopamine content, compared to the sense-treated control (-32%, P>0.05). These data indicate that SRY positively regulates the control of voluntary movement and coordination by regulating dopamine levels in the nigrostriatal pathway in males.

POS-TUE-154

CHARACTERIZATION OF THE KYNURENINE PATHWAY IN MOTOR NEURON: RELEVANCE FOR AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease characterized by progressive loss of motor neurons, resulting in paralysis in most patients and death within 3 to 5 years after disease onset. The underlying cause of the disease is unknown and there is still no rational treatment or therapy. There is strong evidence showing that the kynurenine pathway (KP) is substantially involved in the neurodegenerative processes of ALS. We investigated the involvement of KP in ALS using murine motor neuron cell line (NSC34) and have optimized the culture of human primary motor neurons. Methods: Expression of the KP enzymes was quantified using real-time PCR in 1) IFN-γ treated BV-2 cells (positive control), 2) untreated NSC-34 cells and 3) IFN-γ treated NSC-34 cells. Cells were treated with 100 IU/µl of IFN-γ for 24, 48, 72 hr. The primary human fetal motor neurons were cultured using a new protocol combining centrifugation and density gradient separation processes from fetal spinal cord. Results: The results showed that the overall trend appeared to be a general increase in all the kynurenine pathway enzymes expression in IFN-γ treated NSC-34 cells compared to untreated cells. We have obtained a 90% pure (SMI-32+) primary culture of human motor neurons. Conclusions: We have fully characterized the KP in NSC34. Furthermore, we have successfully optimized a new method to obtain highly purified primary culture human motor neuron. This tool will be very important to study neurotoxicity and neuroprotection to assess therapeutic strategies for motor neuron diseases.

POS-TUE-155

EFFECTS OF COMPLEMENT C5A RECEPTOR INHIBITION IN THE MOUSE PILOCARPINE EPILEPSY MODEL

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Many physiological parameters determine the etiology of seizures and epilepsy, including inflammation. The complement system belongs to the innate immune system and can trigger inflammation. The levels of various complement factors, including the membrane attack complex, have been shown to be increased in animal models of epilepsy and in patients with human temporal epilepsy (Aronica et al., 2007; Neurobiol Dis 26:497ff). Therefore, it is likely that the anaphylatoxin C5a is produced in epileptic brain. Our hypothesis is that activation of complement with the production of C5a plays a crucial role in the pathogenesis of seizures, such as increasing excitability and status epilepticus (SE)-induced cell death. Here, we investigated the anticonvulsant and neuroprotective effects of PMX53, an inhibitor of the C5a receptor CD88, in the pilocarpine seizure model in two different mouse strains. In CD1 mice, injection of PMX53 (3 mg/kg, s.c.) at 1 day and 2 h before pilocarpine injection reduced the number of mice experiencing status epilepticus (SE), defined as more than 30 min of continuous seizures ($p<0.05$, Fisher's exact test), suggesting that PMX53 may be anticonvulsant in this model. In CF1 mice, PMX53 treatment (1 mg/kg, s.c.) at 2, 1 day and 3 h before pilocarpine injection, eliminated pilocarpine-SE-induced mortality, while 22% of vehicle treated mice died ($p<0.05$, Fisher's exact test). Three days after SE, the number of preserved neuropeptide-Y-positive neurons in the hilus of the dentate gryus was doubled in the PMX53-treated mice ($p<0.05$, t-test). In summary, CD88 inhibition may be a novel mechanism that provides anticonvulsant and neuroprotective effects (also see KB abstract at ANS 2011).

POS-TUE-156

ASSESSMENT OF A ROLE FOR ERK SIGNALLING IN THE ANTI-AMYLOIDOGENIC ACTION OF GLYCOSPHINGOLIPID SYNTHESIS INHIBITORS

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Purpose: Inhibition of glycosphingolipid (GSL) synthesis reduces amyloid-beta (Aβ) production in vitro. Previous studies indicate that GSL inhibitors modulate phosphorylation of extracellular signal-regulated kinase 1/2 (ERK) and that the ERK pathway may regulate some aspects of Aβ production. It is not clear if there is causative relationship linking GSL synthesis inhibition, ERK phosphorylation, and Aβ production. **Methods:** Here we treated CHO cells that constitutively express human wild type APP695 with GSL-modulating agents (48 h) to explore this relationship. **Results:** We found that the GSL inhibitor D-threo-I-phenyl-2-hexadecanoylamino-3-morpholino-propanol (D-PPMP) at 5 µM reduced Aβ production by ~50% and significantly suppressed pERK formation, however, the L enantiomer (L-PPMP) that lacks GSL inhibiting activity, also reduced the level of pERK without suppressing Aβ production. Application of MAPK pathway inhibitors PD98059 or U0126 almost eliminated pERK formation with little effect on Aβ. Knockdown of endogenous glucosylceramide synthase, using siRNA, potently suppressed GSL levels while only weakly inhibiting Aβ and pERK formation. Furthermore, induction of GSL accumulation by administration of the acid beta-glucosidase inhibitor, conduritol B epoxide, also suppressed ERK phosphorylation (~50%), but without any obvious impact on Aβ secretion. **Conclusions:** Our data suggest that the alteration in pERK levels associated with the GSL inhibitors is not the principal mechanism by which these drugs inhibit Aβ generation, and that the ERK signalling pathway does not play a crucial role in mediating these anti-amyloidogenic actions.

POS-TUE-157

HISTAMINE H1 RECEPTOR AGONIST AND CONTROL OF OLANZAPINE-INDUCED OBESITY

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The atypical antipsychotic drug olanzapine is widely used to treat the symptoms of schizophrenia, however, it also induces serious metabolic side-effects, such as obesity. An antipsychotic drug's antagonistic affinity to histamine H1 receptors (H1R) is one of the main indicators of weight gain/obesity side-effect. This study aimed to investigate whether a combined treatment of betahistine (a H1R agonist and H3 receptor antagonist) with olanzapine could reduce the body weight/obesity side-effects induced by olanzapine. **Methods:** Female Sprague Dawley rats (n=12/group) were administered orally with either olanzapine (3mg/kg/day, 3 times/day), betahistine (8mg/kg/day), olanzapine plus betahistine (O+B), or vehicle (control) for 2-weeks. **Results:** Rats treated solely with olanzapine exhibited significant body weight gain and increased food intake (all $p<0.001$). However, sole betahistine treatment had no effect on weight gain and food intake. The O+B co-treatment group exhibited significantly reduced feeding efficiency and body weight (~50% net decrease) compared to the sole olanzapine-treated group ($p=0.015$). Olanzapine treatment reduced locomotor activity and increased white fat mass; however sole betahistine had no influence on these parameters. **Conclusion:** These findings revealed that olanzapine-induced body weight gain could partially be reduced by co-treatment with betahistine. Betahistine has H3 receptor antagonistic effects to increase histamine release, which may augment its direct agonistic effects on H1 receptors. These findings further support the important role of H1R in olanzapine-induced obesity, and have important implications for clinical trials using betahistine to control antipsychotic-induced obesity side-effects.

POS-TUE-158

THE INVOLVEMENT OF THE KYNURENINE PATHWAY IN NEUROINFLAMMATION AND NEURODEGENERATION OF MULTIPLE SCLEROSIS PROGRESSION

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Purpose: The kynurenine pathway (KP) has increasingly drawn awareness in multiple sclerosis (MS), for which abnormal levels of KP metabolites have been found. The KP may be involve in neurological deficit from two aspect: firstly, it has been shown that the first rate limiting enzyme of the KP, Indoleamine, 2-3 dioxygenase (IDO) is involve in immune regulation of inflammatory process in the brain; and/or secondly, metabolites from the KP are associated with neurodegeneration. Despite earlier studies showing that the KP may be activated and also the neuroprotective metabolite kynurenic acid (KYNA) production is increased. However, these data do not explain the detrimental effects of the KP in the neuropathology of MS. We hypothesize that this is associated with increased production of the downstream excitotoxin metabolite, quinolinic acid (QUIN). **Methods:** Our studies involve quantifying levels of tryptophan and several KP metabolites in the serum and cerebrospinal fluid of MS patients with early (n=50) and late (n=37) stages diagnosis using HPLC and GC/MS. These patients had not received any recent corticosteroid treatment or other medications known to interfere with the KP at the time of sample collection. **Results:** We found that IDO is upregulated at all the stages of MS compare to healthy controls. We also observed an increased production of excitotoxin, QUIN in MS patients compared to controls implying abnormal alteration to the KP metabolism. Furthermore, we found QUIN to be present in active lesion of human MS postmortem brain tissue suggesting their involvement in neurodegeneration. Interestingly, we found that the profiles of neuroprotective KP metabolites, KYNA and QUIN are unique across various stages of MS progression. **Conclusion:** All the above data suggests the significant involvement of KP metabolism in MS progression and the potential use of these metabolites as biomarkers to assess severity of MS progression.

POS-TUE-159

DEEP BRAIN STIMULATION OF THE POSTERIOR SUBTHALAMIC AREA MODULATES SACCADIC EYE MOVEMENTS

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Deep brain stimulation (DBS) of the caudal zona incerta (ZI) in the posterior subthalamic area (PSA) is being trialled as a refined treatment for movement disorders using a new precise surgical technique. The ZI may influence saccadic eye movements in animals but its role in humans is unknown. **PURPOSE:** To measure the effects of electrical stimulation on head-fixed horizontal predictive saccades in patients with Parkinson's disease or essential tremor. **METHODS:** Patients (n=8) undergoing PSA DBS underwent testing at pre-operative baseline and with four randomized post-operative stimulation settings (nil, 1 volt low frequency stimulation (LFS), 1V high frequency (HFS), and 3V HFS) on day six after surgery. For each condition, 20 trials of 30° saccades were carried out in darkness with infrared video eye movement monitoring and direct current electro-oculography to generate saccade latency, duration, velocity and amplitude data in each gaze direction. **RESULTS:** Saccade velocity was successively reduced by 16.4%, 4.9% and 9.5% by electrode insertion, 1V and 3 V HFS respectively ($p < 0.05$ for each). Saccade duration was similarly increased but there was no change in latency. HFS at 3V reduced saccade amplitude by 16.7% compared to the pre-operative state ($p<0.0001$). LFS (10 Hz, 1V) did not alter saccades. **CONCLUSION:** Saccades had normal latency but were slow and hypometric with PSA DBS. This contrasts with the reduced saccade latency, increased velocity and increased amplitude reported with HFS of the adjacent subthalamic nucleus. We provide the first evidence that human eye movement control structures may lie within the region of the caudal ZI.

POS-TUE-160

A NOVEL VISUAL DISCRIMINATION TASK TO INVESTIGATE THE DORSAL VISUAL FIELD IN RODENTS

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Purpose: The visual system of the rodent is a highly studied brain region, yet many of its fundamental functional characteristics are yet to be described. Here, we detail a novel behavioural task that can be used to measure discrimination at varying dorsal regions of the rodent visual field. **Methods:** A modified version of the aquatic visual discrimination task (Prusky, G.T. et al., 2000) was used. Visual stimuli were projected onto a matte surface at one end of the tank and the surface of the pool was agitated. Groups of male and female mice were first pre-trained to associate a single stimulus with an escape platform in blocks of 10 trials and 2 blocks per day. Upon achieving three consecutive scores ≥80% the mice were then made to discriminate between a vertical and horizontal grating at water level, again associating 1 of the 2 orientations with an escape platform. Upon achieving ≥80% in 4 consecutive blocks of trials, the stimulus was then raised dorsally in increments of 5cm per block. In order for the stimulus to be raised further, mice had to score ≥70% correct per block. Once performance dropped <70% in a given block of trials, the stimulus was lowered to the water level and their discrimination at gradually more dorsal positions was re-tested identically to determine an upper threshold. **Results:** The ability of wildtype mice to correctly discriminate visual stimuli decreased dramatically at stimulus positions exceeding 20 cm above water level. **Conclusions:** Mice have an upper limit in their dorsal visual field at which they can discriminate static visual stimuli above chance.

POS-TUE-161

ACID TREATMENT AS AN ANTIGEN RETRIEVAL METHOD FOR FORMALIN FIXED AND PARAFFIN EMBEDDED HUMAN BRAIN TISSUES

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PURPOSE: Immunohistochemistry is a widely-used technique in neuroscience that enables the study of biomarkers including epigenetic markers within specific brain structures. Formalin and paraffin, commonly used to preserve tissue, can mask epitopes and prevent antibodies from binding to their targets. A number of different protocols for retrieval of antigens have been developed to overcome this problem. A commonly used method is heat-induced antigen retrieval. Two main disadvantages of this technique are that it cannot be applied to fixed cultured cells and that it is difficult to obtain even heating between samples. The purpose of his study is therefore to develop an antigen retrieval method that does not require a heating step. **METHODS:** We specifically looked at staining methylated DNA. High concentration of acid chloride was used to restore immunoreactivity for 5-methylcytosine antibody in normal (N=6) and Alzheimer's disease (N=6) human brain tissues. To test the specificity of the staining, commonly used markers for brain tissue, including the neuronal markers NeuN and MAP2, and the astrocytic marker GFAP were also investigated. **RESULTS:** We found that using a high concentration of acid chloride can re-establish immunoreactivity of our 5-methylcytosine antibody without the heating step. Using antibodies to other nuclear proteins and cytoplasmic markers, we show that this antigen retrieval method can produce specific staining but does not affect all antibodies in the same way. **CONCLUSION:** This protocol reduces the risk of introducing non-specific differences between samples due to the heating step and can be applied to formalin-fixed and paraffin-embedded human brain tissue as well as to paraformaldehyde-fixed cultured cells.

POS-TUE-162

NEURONAL ENDOCYTOSIS OF MULTIFUNCTIONAL POLYMER NANOSPHERES

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Purpose: Multifunctional polymer nanospheres were designed to allow visualisation and release of neuroprotective agents at a neurotrauma injury site. **Methods:** Magnetic and fluorescent nanospheres were assessed for toxic effects on immortalised and primary cell cultures (PC12, rMC-1, hippocampal, cortical neurons) using calcein/ethidium homodimer-1 viability analyses. Endocytosis was assessed using EM, fluorescence microscopy, immunohistochemistry and relaxometry. **Results:** No decrease in cell viability (p>0.05) was observed when nanospheres (up to 250 μg ml^{-1}) were incubated with Ordway PC12 cells, rMC-1 Muller cells, or rat hippocampal or cortical neurons for up to 72 h. EM analysis indicated that the nanospheres entered PC12 cells by multiple mechanisms including macropinocytosis, although clathrin-coated pits and caveolae were not observed. Furthermore, nystatin/progesterone treatment of cultures indicated that caveolin and lipid raft-dependent processes were not required for particle uptake. Once inside the cells, the fluorescent nanospheres aggregated in membrane bound bodies; LAMP-1 immunohistochemistry indicated that these bodies were not lysosomes or late endosomes. Changes in relaxivity reveal a quantifiable compartmentalisation of nanospheres. Additionally, we have observed uptake of fluorescent nanospheres into neurons *in vivo*, following administration at a partial optic nerve injury site. **Conclusion:** The nanospheres we describe may be a useful platform for delivering neuroprotective agents into neurons at neurotrauma injury sites.

POS-TUE-163

DEVELOPMENT OF AN IMPROVED COCHLEAR ELECTRODE ARRAY FOR USE IN EXPERIMENTAL STUDIES

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Purpose: Animal studies play an important role in establishing the safety and efficacy of cochlear implants and the development of new electrical stimulation strategies. In the present study we evaluate the safety and efficacy of a new electrode array designed to more accurately simulate the electrode insertion depths achieved clinically. Methods: The insertion depth and trauma associated with the insertion of a new generation electrode array (Hybrid-L) was compared with a standard experimental electrode array. Each array was inserted into a cat cadaver cochlea (n=6) and a micro-focus X-ray imaged their anatomical location within the scala tympani. The implanted cochleae were then serially sectioned and at every 300 μm they were photographed to determine the position of the array and to examine for insertion trauma. Results: Mean insertion depth for the Hybrid-L array was 334.8° (SD=21°; n=4) versus 175.5° (SD =6°; n=2) for the standard electrode array. This relates to an insertion depth of approximately 10.5 mm and 6 mm respectively. Each electrode array was located in the scala tympani and showed no evidence of electrode insertion trauma. Conclusion: Cochlear's Hybrid-L electrode array can be safely inserted ~50% of the length of the cat scala tympani, placing the tip of the array at approximately the 4 kHz place. This insertion depth is considerably greater than is routinely achieved using a standard array (~12 kHz place). The Hybrid-L array has application in research associated with bilateral cochlear implantation; electric-acoustic stimulation and plasticity studies.

POS-TUE-164

USING OPTOGENETICS TO PROBE NEURONAL CIRCUITRY

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Intro: The advent of optogenetic neuroengineering heralds a new epoch in the investigation of neuronal-circuit function. This technique utilises a range of light-activated proteins with the capacity to bidirectionally control neuronal activity with high spatial and temporal precision. Here we are working to establish the use of optogenetics to probe the neuronal circuitry of the amygdala, which is a region of interconnected nuclei known to be crucial for both the acquisition and storage of emotional memory. **Methods:** Third generation lentiviruses were produced for Channelrhodopsin-2 (hChR2) and Volvox (VChR1) using the calcium phosphate transfection method. Whole-cell patch clamp recordings were used to quantify photocurrent amplitudes from hChR2- and VChR1-expressing HEK293T cells, primary hippocampal cultures and acute slices. LED illumination was used for photorhodopsin-specific excitation. *In vivo* stereotactic lentiviral injections were performed on Wistar rats aged 18-35d. **Results:** Lentivirus titres were calculated in the range of 8x10^{7} IU/mL. Whole-cell peak photocurrent amplitudes ≤ 1nA were recorded in HEK293T cells infected with hChR2(H134R) lentivirus (n=4) and transfected with VChR1 cDNA (n=5). Primary hippocampal cultures infected with hChR2(H134R) yielded peak photocurrents ≤ 800 pA, which were sufficient to drive action potential firing during 10 ms illuminations, tested at frequencies up to 10 Hz (n=4). Acute slices prepared from animals stereotactically injected with hChR2 lentivirus into the auditory cortex demonstrated membrane bound expression after 3 weeks, detectable via fused-fluorophore imaging. hChR2 expression was capable of driving action potentials in infected neurons after 5 weeks recovery (n=2). **Conclusion:** This preliminary work supports the future application of these optogenetic technologies within the amygdala, which in turn will provide new insight into the mechanisms underlying the acquisition and storage of emotional memory.

POS-TUE-165

INVESTIGATION OF SIRNA DELIVERY TO THE MOUSE BRAIN

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The use of RNA interference (RNAi) is being harnessed in nearly every aspect of biomedical research. However, when applying siRNA technology to elucidate the role of novel proteins, success hinges largely on their efficient delivery to targets. The delivery challenge is even greater when the aim is to inhibit the expression of target genes in vivo. To explore this, direct intracerebroventricular (i.c.v.) infusions of 100µl were made into the third ventricle of 8 week old BALB/c mice. Initially, dextran tagged Alexa Fluor 488 was infused (n=6) given it has size (10KDa) and charge properties similar to that of siRNA. Dextran was delivered using an intrathecal catheter attached to an Alzet osmotic mini-pump at a rate of 0.22µl/hr over 2 weeks (500µg). To monitor distribution of such a 'siRNA-like' molecule, tissue was collected and processed for imaging at various time points (day 8, 11, 14). Confocal imaging show gradual and widespread distribution of dextran-Alexa Fluor 488 in regions including frontal cortex, striatum, hippocampus and cerebellum. Subsequently, IGF-IR Alexa-siRNA was delivered (100µg) in a similar manner and compared to untreated controls (n=2). Preliminary western immunoblotting and densitometry analysis show IGF-IR knockdown in the cortex with corresponding decreases in percentage protein. Ongoing imaging will be carried out to establish the distribution of IGF-IR Alexa-siRNA in these brains.

POS-TUE-166

DEVELOPING A HUMAN BRAIN TISSUE MICROARRAY PLATFORM

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Purpose: Tissue Microarray (TMA) is a method for precisely arraying large numbers (10-1000's) of small cylindrical cores of tissue (0.6-2 mm diameter) from multiple brain cases or regions, taken from paraffin embedded tissue blocks, by insertion into a blank paraffin block. We have recently established a human brain TMA facility at The University of Auckland using the Tissue Microarrayer (Advanced Tissue Arrayer, ATA100, Life Sciences). Once constructed, the TMA can be used in histological, immunohistochemical and/or in situ hybridization analysis. Images are acquired using the high-throughput automated Discovery-1 microscope system and analysed by High Content Analysis using automated Metamorph image analysis software. **Method:** To validate this technique a tissue microarray comprising 49 cores from throughout a normal human brain was constructed and analysed immunohistochemically using various antibodies against cell specific markers and disease associated proteins. **Results:** TMA allows for a first pass look at localisation and expression of proteins. For example, the number of cells expressing FUS as well as the expression level was shown to be increased in the Putamen when compared to other regions of the brain in this study. TMA also allows a quick comparison of the type of staining of an antibody. Parvalbumin shows increased neuropil staining in the Thalamus, brainstem and cerebellum throughout the core, whereas the cortex and hippocampus shows predominantly cell body staining. **Conclusions:** This new platform will allow for high throughput, standardized and objective studies to be performed to identify abnormalities in cell morphology and biochemistry in a range of brain disorders as well as the expression and distribution of various proteins and genes in the normal human brain.

POS-WED-001

A PUTATIVE MODULATOR OF THE P75^{NTR} NEUROTROPHIN RECEPTOR REGULATES PERIPHERAL MYELINATION

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Purpose: The neurotrophin family of growth factors play diverse roles in regulating peripheral nerve myelination by Schwann cells. We have recently identified that the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) can either promote or inhibit peripheral myelination, depending on whether it activates neuronally-expressed p75^{NTR} or TrkB receptors. Here we use a putative selective agonist of p75^{NTR} to further examine the role of p75^{NTR} in Schwann cell myelination *in vitro* and *in vivo*. **Methods:** *In vitro* myelination assays were treated with either BDNF or p75^{NTR} agonist, and analysed by Western blot for expression of integral myelin proteins MAG and MBP. P0 SD rat pups were injected along the sciatic nerve with either BDNF, p75^{NTR} agonist, or vehicle. Injections were repeated 48 hours later. Sciatic nerves were analysed for myelin formation as described above, 48h after the second injection. **Results:** BDNF treatment resulted in a two-fold increase in both MAG and MBP expression *in vitro* (n=2), however it did not appear to significantly affect MBP levels *in vivo* (n=2). The p75^{NTR} agonist caused a concentration-dependent increase in the expression of both MAG and MBP *in vitro*, with a maximal 3-fold increase (n=2). This result was reflected *in vivo*, where the p75^{NTR} agonist also gave a 3-fold increase in the expression MBP in sciatic nerve (n=3). **Conclusion:** A putative p75^{NTR} agonist increases peripheral myelination both *in vitro* and *in vivo*, suggesting there is merit in pursuing p75^{NTR} activation as means of promoting peripheral myelin repair.

POS-WED-002

DEVELOPMENT OF THE ADOLESCENT RAT PRE-FRONTAL CORTEX – A GOLGI STUDY

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Adolescence is a period during which the cerebral cortical gray matter decreases in volume. Rats are commonly used to model both structural and functional aspects of human adolescent brain development but little is known about development of the neuronal dendritic tree during adolescence. Purpose: To quantify the dendritic structure of Layer II/III pyramidal neurons in the prefrontal cortex of the rat during adolescence. Methods: On postnatal day 20, 28, 41, 52 and 90, Long Evans rats (n=4) were perfused and brain tissue was immersed in Golgi solution. When fully impregnated, serial 200µm thick sections were cut and processed with the Golgi method. Fully impregnated layer II/III neurons were randomly selected and traced using a drawing tube attached to a light microscope. Sholl analysis was used to determine total dendritic length, number and type of branches and spine density on terminal dendrites. Data was analysed using ANOVA, followed by post hoc Newman-Keuls test. Results: There was no significant effect of age on length, number of branches or bifurcations of the total dendritic tree. However in the basal dendritic tree age had a significant effect on dendrite length ($F(4,56) = 17.05$) ($p<0.0001$); number of 1st, 2nd and 3rd order dendritic branches ($F(4,55) = 2.119$) ($p<0.001$); number of spines per unit length of distal dendrite ($F(4,108) = 9.042$) ($p<0.0001$). Conclusions: Significant changes occur in the dendritic arbor of Layer II/III pyramidal neurons in the pre-frontal cortex of the rat during adolescence. The networks of the mature cerebral cortex are finalized during adolescent brain development, thus perturbation of this process may result in aberrant connections and consequent long-term changes in brain structure and function.

POS-WED-003

EPHA4 INHIBITS NEURAL PRECURSOR ACTIVITY IN THE ADULT HIPPOCAMPUS

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Purpose: The production and activation of neural precursors in the adult hippocampus is functionally important for learning and memory. Here, we explore the effect of EphA4, which has previously been shown to regulate neural precursor survival in the subventricular zone, on precursor activity in the adult mouse hippocampus. **Methods:** We used the hippocampal neurosphere assay to test neural precursor activity *in vitro*. In addition, immunohistochemical analysis was undertaken to determine proliferating cell (BrdU-labelled) and immature neuron (doublecortin-positive) densities in the hippocampus of adult EphA4 knockout (EphA4$^{-/-}$) and kinase-dead (EphA4$^{KD/KD}$) transgenic mice. **Results:** Hippocampal cells from EphA4$^{KD/KD}$ (n=8) and EphA4$^{-/-}$ (n=3) mice gave rise to a 2.5-fold and 3-fold increase in precursor activity, respectively, compared to wild-type controls (n=11). We observed 5.2 ± 0.3 BrdU-labelled cells/mm of the dentate gyrus in the EphA4$^{KD/KD}$ hippocampus (n=6 animals), 5.4 ± 0.1 cells/mm in the EphA4$^{-/-}$ hippocampus (n=4), but only 4.6 ± 0.2 cells/mm in the control (n=10). We also observed over 20% more doublecortin-positive cells/mm of the dentate gyrus in both the EphA4$^{KD/KD}$ (n=6) and EphA4$^{-/-}$ (n=6) mice, than in wild-type littermates (n=12). Furthermore, we were able to achieve an increase in neural precursor activity through antagonism of the EphA4 receptor, with an 89.5 ± 19.4% increase in activity following treatment with a recombinant protein antagonist, EphA4-Fc (n=5), and an 80 ± 14% increase in activity following treatment with an antibody antagonist (n=6). **Conclusion:** These results indicate that EphA4 exerts an inhibitory effect on precursors in the adult mouse hippocampus, although the mechanism by which this occurs needs further investigation.

POS-WED-004

SPINAL CORD INJURY-INDUCED CHANGES IN THE PROTEOME OF THE DEVELOPING *MONODELPHIS DOMESTICA*

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AIM: Trauma to the spinal cord (SC) detrimentally affects normal motor, sensory and autonomic functions. Monodelphis domestica is an excellent developmental model of spinal cord injury as it was demonstrated that following spinal transection in the first two weeks of life a re-growth of axons across the site of injury can occur but this ability declines with age (Lane et al, 2007). **METHODS:** Newborn pups were subjected to complete SC transection under isofluorane anesthesia at either postnatal (P)7 or P28. Segments of the cord caudal to injury were collected one or seven days later for proteomics analysis. Cords were homogenized, proteins extracted and separated first according to their isoelectric point followed by molecular weights. Intensity of protein bands was analysed by densitometry and changes of +-0.5 relative to age matched controls were sent for identification by mass spectrometry. **RESULTS:** Out of 57 proteins identified by mass spectrometry >50% were cytoplasmic and 70% of these belonged to families of proteins with binding properties. Other identified proteins were involved in various biological functions such as regulation (40%), metabolism (26%), inflammation (19%) and structure (15%). Following injury at P7 more proteins were down- than up-regulated. The opposite was true for P28 injured cords. **CONCLUSIONS:** Changes in the proteome in response to injury were age dependent. More changes were detected after one than seven days after injury at both ages. More proteins were down-regulated at P7 and more were upregulated at P28. Response to injury appears to be multifactorial.

POS-WED-005

ENVIRONMENTAL ENRICHMENT AND REDUCED INHIBITION IMPROVES REINNERVATION AND FUNCTIONAL OUTCOMES AFTER CEREBELLAR LESIONS

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Purpose: Long-term improvement following neurotrauma requires either re-growth of damaged connections or modifications in remaining ones, so that rebuilt neural circuits restore function. Ephrin-A guidance cues contribute to the inhibitory cellular environment that prevents axonal growth following lesion. We have used ephrin-A knockout (KO) mice, to study axon collateral reinnervation in a less inhibitory cellular environment. Given that loss of ephrins may reduce accuracy of re-established connections, we tested whether psychomotor enrichment could compensate for the loss of guidance molecules and promote accurate circuit repair. Methods: Adult WT and KO mice (4-12 per group) received unilateral section of an olivocerebellar path (pedunculotomy, Px) and were returned to standard (std) or enriched (EE) housing for 8 weeks before assessment of behaviour (accelerating rotarod) and olivocerebellar reinnervation (VGLUT2 immunohistochemistry). Results: Intact WT and KO mice performed equally well on the rotarod, with minimal effects of enrichment. Following Px, WT-std and KO-std mice performed significantly worse compared to intact animals ($p<0.05$) and KO-Px-Std mice were worse than WT-Px-std ($p<0.01$). WT-PxEE mice were only marginally better than WT-Px-std ($p>0.05$), but KO-PxEE mice performed significantly better than KO-Px-Std ($p<0.001$) almost reaching the level of intact KO mice. Furthermore, EE induced a small amount of reinnervation in WT-PxEE and significantly more reinnervation in KO-PxEE mice ($p<0.001$). Conclusions: Removal of inhibitory proteins resulted in a higher amount of reinnervation in association with better behavioural recovery. This suggests that while treatments that reduce inhibition in the brain may promote axonal growth, their combination with psychomotor feedback offsets the compromised accuracy to provide genuine circuit repair.

POS-WED-006

RETINAL GANGLION CELL TYPES IN HIGH ACUITY REGIONS OF THE PIGEON RETINA

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Purpose: The pigeon retina contains two retinal specializations for high acuity vision: the fovea and the *area dorsalis* (AD). Both regions contain a similar density of retinal ganglion cells (RGCs); however there is limited knowledge regarding the complete array of RGC types present in the fovea and AD. The aim of this study was to identify RGC types that might mediate high acuity in the fovea and AD. **Methods:** RGCs (n = 669) were labeled using a Diolistics approach and reconstructed with a confocal microscope. Soma size, dendritic field size, branching pattern, stratification and eccentricity were measured and the RGCs classified based on these parameters. **Results:** The pigeon retina contains 12 identifiable types of RGCs all of which are localized to the AD with eight of these types also present in the fovea. Dendritic field diameter in the AD ranged from 14-269 µm, with each group having a characteristic size. In the fovea, the range of dendritic field diameters was smaller (31-81 µm). Where the primate and pigeon retina contain RGC types which exhibit increasing dendritic field size with eccentricity from the fovea, some pigeon RGC types showed the reverse. This was also true of the AD. **Conclusions:** Of the 12 different types of pigeon RGCs, the smallest dendritic field diameter observed in our sample was approximately twice the size required to underlie pigeon visual acuity (12 cpd). Unlike the mammalian RGC distribution where dendritic field diameter increased proportional to the eccentricity from a specialized region, it appears that the avian retina with two high acuity regions may have two independent systems mediating high visual acuity.

POS-WED-007

THE DE-UBIQUITYLATING ENZYME USP9X IS ESSENTIAL FOR NORMAL CNS DEVELOPMENT IN MOUSE

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Purpose: The ubiquitin system plays an important role during neurogenesis. Defects within this system have been linked to a range of neurodegenerative diseases. Neural stem cells (NSCs) have potential for use in cell transplantation therapies and as cellular models for disease. The substrate-specific de-ubiquitylating enzyme USP9X is enriched in NSCs and is highly expressed throughout the developing CNS. USP9X has multiple substrates, many playing important roles during neurogenesis. This study aims to analyse the role of USP9X during neural development using the mouse as a model. **Methods**: The Cre/loxP recombination system is used to conditionally delete USP9X in the developing CNS. Two models are utilised: Nestin-Cre deletion of USP9X in the whole CNS and Emx1-Cre delelting USP9X in the forebrain only. USP9X is deleted in NSCs prior to the onset of differentiation allowing the examination of effects on NSCs, neurons and glia. **Results**: Nestin-Cre mediated deletion of USP9X resulted in early postnatal (P1) lethality. At E18.5 (n = 5) and P0 (n = 3) mice display reduced axonal processes projecting from the entorinal cortex to the hippocampus. Emx1-Cre mediated deletion of USP9X allowed mice to survive into adulthood. In the adult (7 weeks) we observed a dramatic reduction in the size of the corpus callosum and the hippocampus (n = 4). **Conclusion**: USP9X is essential for normal CNS development in mouse. The reduction in corpus callosum size in the adult and the reduced axonal processes observed in earlier stages suggest USP9X is essential for normal axon elongation. We are currently analysing the mechanisms by which loss of USP9X induces this phenotype.

POS-WED-008

THE ROLE OF EPHRINB1 IN THE DEVELOPMENT, MAINTENANCE AND PLASTICITY OF THE MARMOSET MONKEY GENICULOSTRIATE PATHWAY

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Purpose: This project investigates the role of ephrinB1 in the development of the geniculostriate pathway in the nonhuman primate, as well as following lesions of neonate and adult V1. **Methods:** The spatiotemporal expression of ephrinB1 was examined immunohistochemically in the marmoset monkey visual system at postnatal day (PD) 0, PD14, PD30, PD90 and adult (n=1 each). V1 ablation surgeries were performed on neonates (PD14) with short (3 weeks; n=2) or long-term (>1year; n=2) recovery periods and adult (>1year; n=1). Expression profiles following V1 lesions were compared against the normal lesion age and age-matched controls. Laminar expression in V1 was determined using optical density analysis and cell counts were performed on the superior colliculus (SC). **Results:** From PD0 to PD30, we observed synaptic ephrinB1 expression in the visual cortex that was restricted to V1, in layers with direct geniculate connectivity (3Bβ, 4 and 6) and sustained in layer 4 in the adult. The geniculate nucleus (LGN) demonstrated sustained upregulation of expression, in contrast to the decreasing ephrinB1 positive cell densities observed in the non-thalamic SC throughout postnatal development ($p<0.0001$). We also report ephrinB1 expression by glutamatergic and GABAergic neurones and oligodendrocytes in the marmoset visual system. Following neonatal V1 ablation, we observed ephrinB1 upregulation in layer 3C of contralateral V1 and expression in ipsilateral V2 after short and long-term recovery respectively and upregulated expression by oligodendrocytes in the lesion site following adulthood V1 lesion. **Conclusion:** Our results suggests that ephrinB1 plays a multifunctional role involving the establishment and maintenance of thalamocortical connectivity and plasticity, and could be implicated in assisting damaged connections following neonatal and adulthood lesions to V1.

POS-WED-009

TEN_M3: ROLES IN THE DEVELOPMENT OF THE RODENT STRIATUM

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Ten_m3 is a transmembrane glycoprotein which regulates cell adhesion and axonal guidance during development. We found that Ten_m3 is expressed in patches within a subregion of the striatal matrix compartment. To examine a potential role for Ten_m3 in the guidance and targeting of thalamostriatal axons, one of the major sources of input to the striatum, stereotaxic injections of biotinylated dextran amine were made into the parafascicular thalamic nucleus (PFN) in adult mice. The area occupied by parafascicular terminals was increased in KOs, although this change was not significant (p=0.084, t-test). Interestingly, however, terminals were significantly denser in KOs than in WTs (WT: 124.21 ± 9.07 (arbitrary units, mean ± SEM), n = 9; KO: 166.66 ± 11.58 (arbitrary units, mean ± SEM), n = 5; p = 0.018, t-test). Qualitatively, this change was associated with a more uniform distribution of terminals in KOs, compared to a more 'patchy' arrangement in WTs. Moreover, these differences were observed in the absence of a significant change in PFN area and cell number between genotypes. Preliminary experiments using DiI crystals to trace thalamostriatal projections in fixed pre- and postnatal brains aim to further investigate this novel role for Ten_m3 in directing thalamostriatal axons by examining the developmental timeline and patterning of these projections in mice. Data from WT mice suggest that thalamic axons reach the striatum by embryonic day 17-18. The targeting of these terminals to the matrix and their unique 'patchy' arrangement is evident by birth, coincident with the time that Ten_m3 becomes apparent in the matrix. These data suggest a role for Ten_m3 in the formation of the thalamostriatal projection.

POS-WED-010

TEMPORAL EXPRESSION OF CONNEXINS DURING NEURONAL DIFFERENTIATION OF NT2/D1 CELLS

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Purpose: Gap junctions are known to be involved in growth and development, however the roles and regulation of specific connexin (Cx) subtypes in neuronal differentiation are not well understood. Changes in Cx expression may be involved in proper development of neurons, thus we have profiled the temporal expression of three neural Cxs during neuronal differentiation of NT2/D1 cells. **Methods:** Differentiation was induced via 4 weeks treatment with 10 μM retinoic acid (RA), followed by 2 weeks mitotic inhibitor treatment. Protein and mRNA were isolated from mixed cultures, in triplicate, at weekly time-points during RA treatment, as well as from undifferentiated NT2/D1 cells and differentiated hNT neurons, and Cx expression assessed using immunoblotting and RT-PCR. **Results:** Cx43 protein was abundant in NT2/D1 cells. Cx43 protein was down-regulated five-fold by day 3 of RA treatment, relative to undifferentiated cells, remaining at this low level throughout RA treatment and was negligible in hNT neurons. Interestingly, Cx43 mRNA was highly expressed for the first 7 days of RA treatment before being downregulated by approximately half by day 14, indicating post-transcriptional regulation of Cxs was altered during differentiation. Cx30 and Cx36 mRNA were expressed at low levels in undifferentiated NT2/D1 cells. Cx30 mRNA was upregulated four-fold during RA treatment by day 14 before decreasing to two-fold of baseline expression by day 21. Cx36 mRNA expression increased two-fold and remained elevated at this level throughout the remaining RA treatment. **Conclusion:** We observed a tight regulation of Cx subtype expression during differentiation. The upregulation of Cx30 and Cx36 suggests these subtypes may be necessary for neuronal development, while Cx43 may be important for maintenance of an undifferentiated state.

POS-WED-011

DEVELOPMENT OF FULL WEIGHT SUPPORTED STEPPING AFTER COMPLETE SPINAL CORD TRANSECTION IN THE NEONATAL OPOSSUM

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Complete spinal cord injury (SCI) usually results in permanent loss of motor and sensory functions below the level of the lesion. Previous work in neonatal opossums demonstrated regrowth of axons and functional recovery occurs following SCI, but these abilities decline with age (Lane et al., Eur. J. Neurosci., 25, 2007). METHODS: This study used the opossum *Monodelphis domestica*. At post-natal day (P) 7 or P28 (8-9 per group) the mid thoracic spinal cord was completely transected under surgical conditions. Three months later behavioural tests, including BBB, swimming and treadmill, were used to analyse locomotion and gait of the animals. Following these tests, brainstem neurons whose axons passed through the injury site were fluorescently labeled, using Fluororuby injected below the level of injury, counted and mapped in the brainstem. Fixed cord segment incorporating the injury site was sectioned and stained for myelin, neurofilament and neuron specific enolase. RESULTS: P7-injured opossums grew an axonal bridge across the injury site. Fluorescent retrograde labelling confirmed that some of these axons were descended from brainstem neurons and some were of spinal origin. This growth was accompanied by improved locomotion (BBB score=16±1.2) and an ability to swim using hind limbs. P28-injured opossums had neither supraspinal nor spinal neurons crossing the injury site yet they walked using full weight supporting footsteps and appeared to have some degree of forelimb-hindlimb coordination (BBB score=12±0.2); however, they did not move their hind limbs during swimming. Gait analyis was performed using the treadmill (6m/min). Interlimb coordination assessment showed that control and P7-injured animals walk with full coordination, but with different paw placement timings, but P28-injured animals were poorly coordinated. CONCLUSION: We have shown that animals can perform well at locomotor testing in the complete absence of supraspinal input, but results from the swimming test and gait analysis studies can differentiate between different degrees of supraspinal innervation and local spinal circuitry.

POS-WED-012

DARK REARING ENLARGES RECEPTIVE FIELDS BUT DOES NOT AFFECT FUNCTIONAL TOPOGRAPHY IN EPHRIN-A2/A5 KNOCKOUT MICE

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Purpose: The visual world is represented topographically within the superior colliculus (SC) maintaining the strict spatial order present in the retina. Numerous mechanisms establish this topography during development including ephrin-A guidance ligands, patterned retinal activity and visual experience. Previous studies indicated ephrin-A2/A5 knockout (KO) mice have disrupted retinotectal projections and ectopic termination zones within the SC. Mice lacking spontaneous retinal activity have diffuse arbours with normal topography, while mice lacking both ephrinAs and spontaneous retinal activity lack order altogether. However, it's not known the extent to which visual experience acts with ephrin ligands to establish topography. **Methods:** KO and wildtype (WT) mice were dark reared (DR) or raised in standard conditions (LR) from birth for 8 weeks. Topography of the retinotectal projection was mapped by multiunit electrophysiological recording in the SC. **Results:** Ectopic points were recorded in all DR KO mice (11%), and LR KOs (20%), though none were present in WT mice. Additionally, whilst topography was disrupted in DR KOs (0.020, rostral-caudal distortion value), it was not worse than LR KO mice (0.022); both DR and LR KO mice were worse than WT mice (DR, 0.011, p=0.04; LR 0.013, p=0.02). Receptive field size was increased in DR KO (5.6cm^2) and DR WT mice (5.45cm^2) compared to LR KO (3.8cm^2; p=0.05) and LR WT mice (2.59cm^2, p=0.001) respectively. There was no difference between DR KO and DR WTs. **Conclusion:** Our results suggest visual experience isn't a major factor in establishing topography of the retinotectal projection, but that it is necessary for the refinement of receptive fields, and does so independently of ephrin-A2/A5.

POS-WED-013

IDENTIFYING THE MOLECULAR SIGNALS REGULATED BY THE UNPROCESSED BDNF

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The neurotrophin family of growth factors is essential for development and maintenance of the peripheral nervous system (PNS), as well as exerting important influences upon additional processes such as dendritic arborization, synaptic transmission, and myelination. The recent discovery that the unprocessed precursor forms of the neurotrophins (proneurotrophins) promote death 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 show that proNGF and proBDNF have no significant effect on Schwann cell proliferation or differentiation in vitro (n=4), and utilising in vitro NFκB luciferase reporter assays (n=4) show that neither proNGF or proBDNF significantly increase NFκB activation over that seen with the mature forms in cultured Schwann cells. Utilising Live/Dead viability assays on freshly isolated Schwann cells (n=4), we have established that both proNGF and proBDNF induce low levels of Schwann cell death in a concentration and time-dependent manner. Taking a global approach to further interrogate proBDNF signalling we have used microarray technology (n=3) and have identified several key downstream signalling pathways differentially regulated by proBDNF in Schwann cells. In particular, proBDNF significantly influences the expression of key cholesterol biosynthesis enzymes in Schwann cells, as well as key molecules involved in myelination. We are currently utilising a variety of techniques, including in vitro myelination and cholesterol assays, to validate these observed changes and elucidate the mechanisms involved in this proBDNF-mediated signalling. These findings suggest an important additional role for proBDNF in regulating the availability of cholesterol for incorporation into peripheral nervous system myelin.

POS-WED-014

SEX- AND REGION-SPECIFIC DIFFERENCES IN BDNF-TRKB SIGNALLING IN THE HIPPOCAMPUS OF BDNF AND 5-HT1A RECEPTOR MUTANT MICE

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Purpose: Dysregulation of brain-derived neurotrophic factor (BDNF) and 5-HT1A receptors has been implicated in schizophrenia and depression. Here, we explore how 5-HT1A receptor knockdown may influence BDNF expression and signalling in the dorsal (DHP) and ventral hippocampus (VHP). **Methods:** We compared male and female double mutant mice (5-HT1A-/-/BDNF+/-) with single mutant (5-HT1A-/-, BDNF+/-) and wildtype controls. Protein expression of BDNF, its receptor TrkB and phosphorylation of TrkB (pTrkB) were examined using Western blot analysis (n=5-6 per genotype). **Results:** In male mice, the BDNF+/- genotype resulted in the expected ~50% reduction of BDNF levels in both DHP and VHP. However, only in DHP there was also a significant increase in pTrkB/TrkB ratios. These changes were independent of the presence or absence of 5-HT1A receptors. In female mice, as expected, BDNF levels were reduced in BDNF+/- and 5-HT1A-/-/BDNF+/-, but in contrast to male mice there was also an intermediate reduction in 5-HT1A-/- compared to wildtype controls. Moreover, and again in contrast to male mice, female BDNF+/- mice (irrespective of the 5-HT1A genotype) showed an approximately three-fold increase of pTrkB levels in the VHP, but not DHP. There were no significant changes in TrkB levels in any of the groups. **Conclusion:** The BDNF+/- genotype induced markedly sex-specific regional differences in BDNF signalling. However, 5-HT1A receptor knockout had only limited effects on BDNF levels or signalling. These results may shed more light into sex differences in schizophrenia and affective disorders and functional differences between the DHP and VHP.

POS-WED-015

OPPOSING ROLES FOR TEN-M2 AND TEN-M4 IN THE DEVELOPMENT OF IPSILATERAL RETINAL PROJECTIONS

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We have previously demonstrated that the transmembrane glycoprotein Ten-m3 plays a critical role in formation of the binocular visual pathway. Here we have investigated potential roles for other members of the Ten-m family in this process. Expression studies revealed that Ten-m2 and Ten-m4 are both present in retina, dLGN, and visual cortex. Anterograde tracing of retinal projections using cholera toxin B showed that ipsilateral retinal projections occupied a smaller area of dLGN in Ten-m2 knockouts (n=10) than in WTs (n=12), being significantly different only within rostral dLGN ($p<0.05$, Multivariate ANOVA). In Ten-m4 KOs (n=12), ipsilateral projections occupied significantly more of the dLGN, but this difference was limited to caudal dLGN ($p<0.05$, Multivariate ANOVA). Similar changes were found in the superior colliculus. To isolate the source of these defects, retrograde tracing was performed by injecting wheat-germ agglutinin conjugated to horse-radish peroxidase into dLGN of WT (n=5) and KO mice. In Ten-m2 KOs (n=5), there was a significant decrease in the area and number of ipsilaterally-projecting retinal ganglion cells ($p<0.05$, Student's t-test). Retrogradely-labelled cells were absent specifically from ventral retina. In Ten-m4 KOs (n=6), the number and area of ipsilaterally-projecting cells was increased into dorsotemporal retina ($p<0.05$, Student's t-test). These changes are not dependent on levels of retinal Zic2 expression – a transcription factor which is a critical determinant of ipsilateral cell fate. While we are currently looking into the mechanism and functional consequences of these deficits, our data suggest that Ten-m2 and Ten-m4 exert opposing influences on the specification and/or guidance of ipsilateral retinal projections.

POS-WED-016

AAV VECTOR-MEDIATED OVEREXPRESSION OF CB1 CANNABINOID RECEPTOR IN PYRAMIDAL NEURONS OF THE HIPPOCAMPUS PROTECTS AGAINST SEIZURE-INDUCED EXCITOXICITY

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The CB1 cannabinoid receptor is the most abundant G-protein coupled receptor in the brain and a key regulator of neuronal excitability. **Purpose:** Here we investigated whether increased CB1 gene dosage in glutamatergic hippocampal neurons has therapeutic effects in seizure-induced pathogenesis. **Methods**: A transcriptional Stop-element flanked by loxP sites was cloned into an adeno-associated virus (AAV) expression cassette between the β-actin promoter and the cDNA encoding GFP or CB1 to obtain pAAV-Stop-GFP or pAAV-Stop-CB1. Recombination was validated in transfected HEK cells. AAV-Stop-CB1 vectors 1 µl ($7x10^7$ viral genomes) were injected bilaterally into the dorsal hippocampus of 3 months old wt mice (AAV-WT) and transgenic mice expressing Cre recombinase exlusively in glutamatergic neurons (AAV-Glu-CB1). The severity of kainic acid (KA)-induced (30 mg/kg; i.p.) acute epileptiform seizures was monitored for two hrs and brains collected after 5 days. Neuronal degeneration was determined by FluoroJade staining. **Results:** Recombination of AAV genomes was strictly limited to Cre expression in vitro and in vivo. Immunoblot analysis showed an 2.5-fold increase ($p<0.001$; unpaired t test) of CB1 receptor protein in hippocampi of AAV-Glu-CB1 (n=3) compared with AAV-WT (n=3) mice. The seizure score was decreased ($p = 0.0007$, Mann Whitney test) and survival improved (Kaplan-Meier analysis; $p = 0.0494$, log rank test) in AAV-Glu-CB1 mice (n=11) compared with controls (n=15). KA-induced neuronal damage was attenuated in the hippocampus of AAV-Glu-CB1 mice but not in AAV-WT mice ($p<0.05$, unpaired t-test). **Conclusion:** Increased CB1 signaling in pyramidal hippocampal neurons acts as a safeguard against the adverse effects of excessive excitatory network activity.

POS-WED-017

ACTION POTENTIAL OUTPUT MODULATION IN THE FIRST NODE OF RANVIER

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Purpose: The axon initial segment (AIS) and first node of Ranvier are both sites with a high Na^+ channel density, low threshold for action potential (AP) generation and are positioned within ~50 and 100 µm distance from the cell body, respectively. All fast Na^+ channel-mediated action potentials initiate in the AIS but it is not well understood how the first node of Ranvier contributes to spike generation. **Methods:** Thick-tufted layer 5 (L5) pyramidal neurons were studied in rat cortical brain slices using whole-cell patch-clamp recording with intracellular Alexa Fluor 594 and biocytin. To visualize and target the first node we used either one- or two-photon fluorescence excitation illumination and post-hoc morphological reconstructions. **Results:** In L5 neurons the first branch point, a marker of the node, was located ~130 µm distance from the soma and generated 2 – 3 major axon collaterals (n = 26). Somatic current injections in L5 neurons without a node, due to the slice cutting, generated regular spiking patterns (~10 Hz, n = 24) whereas neurons with longer axons showed both regular- and burst-firing responses (>200 Hz, n = 27, $P < 0.002$). Furthermore, the elimination of the first node by using two-photon laser axotomy induced a selective reduction in spike output gain and increased the action potential voltage threshold during depolarization (n = 6). These data were consistent with focal Na^+ channel inactivation at the first node (2 µM TTX, n = 8). **Conclusion:** The first node of Ranvier is not only essential for re-excitation of the propagating action potential but also significantly modulates the spike output rate and pattern, thereby contributing to encoding of synaptic inputs.

POS-WED-018

ALFAXALONE CAUSES DOSE-DEPENDENT BIPHASIC ALTERATIONS IN SYNAPTIC INHIBITION OF RAT HYPOGLOSSAL MOTONEURONS

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Purpose: The neurosteroid alfaxalone is a commonly used injectable anaesthetic agent in laboratory and veterinary anaesthesia. A dose response study on the effects of alfaxalone on synaptic inhibition of motoneurons was conducted. Methods: Whole cell patch clamp recordings were made from visualized hypoglossal motor neurons (n=9) in transverse brainstem slices (300 micron thickness) made from 10 to 12 day old Wistar rats anaesthetized with sodium pentobarbitone (100 mg/kg i.p.). Spontaneous and evoked inhibitory postsynaptic currents (IPSCs) were recorded at a holding potential of -60mV using a CsCl-based internal solution in the presence of non-NMDA and NMDA glutamate receptor blockers NBQX (10uM) and DL-APV (50uM). Alfaxalone was bath applied at 10 nM, 30 nM, 100 nM, 300 nM, 1 uM, 3 uM and 10 uM concentrations. IPSC peak amplitude, spontaneous IPSC interval, half-width, 10-90% rise time and paired pulse ratio (interstimulus interval 200 ms) were measured. Results: Spontaneous IPSC amplitude decreased in a dose-dependent manner; mean amplitude at 1uM was 84.77±6.85% (mean±SEM) of control. The spontaneous IPSC interval increased from 0.99±0.18 (control) to 1.75±0.47 seconds (176.7±47.4%) at 1uM and further increased to 4.68±1.11 seconds (473±112%; $P<0.05$) at 10 uM. Evoked IPSC amplitude showed progressive dose-dependent reduction up to 1uM concentration, but paradoxically increased at higher concentrations. At 1uM, evoked IPSC amplitude, half width and 10-90% rise time were 58.3±6.8%, 124.9±5.7% and 154.7±19.6% of control respectively ($P<0.05$). Paired pulse ratio was not significantly altered by alfaxalone. Conclusion: Alfaxalone caused a dose-dependent decrease in synaptic inhibition at concentrations up to 1uM but at higher doses may enhance synaptic inhibition.

POS-WED-019

REGULATION OF SYNAPSE FUNCTION BY SYNAPSE-ASSOCIATED PROTEIN 97 ISOFORMS

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Purpose: Synapse-associated protein 97 (SAP97) is one of the members of the membrane-associated guanylate kinase (MAGUK) family of scaffold proteins. Alternative splicing of SAP97 transcripts gives rise to palmitoylated alpha SAP97 and L27-domain containing beta SAP97 isoforms. Long-term potentiation (LTP) and depression (LTD) are the two major forms of synaptic plasticity that are expressed in the hippocampus and are thought to form the cellular basis of learning and memory. **Methods:** To elucidate the role of SAP97 isoforms in synaptic plasticity, we used electrophysiology, whole-cell patch-clamp paired recordings and puff drug application to test synaptic and surface NMDAR-mediated EPSCs and AMPAR-mediated EPSCs in hippocampal neurons. **Results:** We find and beta forms of SAP97 prevent LTP but enhance LTD via−that both alpha independent isoform-specific mechanisms. we show that alpha SAP97 occludes LTP by enhancing the levels of postsynaptic AMPARs, while beta SAP97 blocks LTP by reducing the synaptic localization of NMDARs. Examination of the surface and synaptic pools of AMPARs and NMDARs before and after plasticity indicates that alpha SAP97 selectively regulates the synaptic pool of AMPARs, whereas beta SAP97 regulates the extrasynaptic pools of both AMPARs and NMDARs. Knockdown of beta SAP97 was found to rescue the synaptic localization of both AMPA and NMDARs, showing the endogenous beta SAP97 restricts glutamate receptor expression at excitatory synapses. **Conclusion:** Our data support a model wherein SAP97 isoforms can regulate the future ability of synapses to undergo plasticity by controlling the surface distribution of AMPARs and NMDARs.

POS-WED-020

CONTRIBUTION OF NR2B- AND NR2D-CONTAINING NMDARS TO DIFFERENT FORMS OF LTP AND THEIR LOCATION AT THE SYNAPSE

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In area CA1 of the hippocampus, varied forms of long-term potentiation (LTP) have been shown to coexist, each involving different intracellular signalling and effector cascades. Most forms of LTP are dependent on activation of postsynaptic NMDARs, however controversy exists over the relative roles of receptors containing different NR2 subunits. We have investigated the involvement of NMDARs containing the NR2B and NR2D subunit in different forms of LTP at the CA3-CA1 synapses in hippocampal slices from male Wistar rats (7-8wks). The selective NR2B antagonist RO 25-6981 (1µM) had no effect on short- and long-lasting LTP induced by 1 and 8 trains of theta-burst stimulation, respectively (1TBS, n=6; 8TBS, n=5) but dramatically reduced the magnitude and persistence of an intermediate LTP induced by 4 TBS (n=9, $p<0.01$). UPB141 (3µM), a selective antagonist of the NR2D subunit did not affect 1 TBS induced LTP but severely attenuated LTP induced by 4 and 8 TBS (4TBS, n=5, $p<0.01$; 8TBS, n=5, $p<0.01$). To assess the location of NR2B- and NR2D-containing NMDARs, isolated NMDA fEPSPs were recorded and glutamate spill-over was enhanced by delivering a 5-pulse burst at 100Hz. RO 25-6981 had no effect (n=9) but UBP141 significantly reduced these synaptic burst responses (n=6, $p < 0.01$). Inversely, after inhibiting synaptic NMDARs with the use-dependent channel blocker MK-801 (10µM) RO 25-6981 significantly reduced the burst-induced response (n=5, $p<0.01$) whereas UBP141 showed no effect (n=8). In summary these data show that various forms of LTP require the activation of different NMDAR NR2-subunits and that NR2B receptors are predominantly located extrasynaptically while NR2D receptors are primarily found at the synapse.

POS-WED-021

HISTONE ACETYLATION REGULATES LTP IN THE INFRALIMBIC MEDIAL PREFRONTAL CORTEX

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Histone modification, including histone acetylation, plays an important role in regulating gene transcription. Furthermore, formation of long-term memory depends on protein synthesis in target cells. We have found that histone acetylation in the infralimbic medial prefrontal cortex (ILPFC) is required for the consolidation of fear extinction. We therefore tested if histone acetylation is required for synaptic plasticity in the ILPFC by looking at LTP, an underlying mechanism for memory formation. Field recordings were made in layer 5 of acute brain slices from C57B6 mouse ILPFC. A bipolar stimulation electrode was placed in layer 2/3. Tetanic stimulation readily evoked LTP at these inputs (120.5±6%, n=5). Inhibition of PCAF, a protein that binds to histone acetyltransferases to allow histone acetylation, blocks the formation of LTP (83.5±13.5%; n=4; $p<0.05$). These results suggest that memory formation for fear extinction requires LTP at synapses within the ILPFC. Consistent with the behavioural studies, we conclude that histone acetylation is necessary for long-term synaptic plasticity within the ILPFC.

POS-WED-022

STAUFEN2 REGULATES MAP1B mRNA DISTRIBUTION IN HIPPOCAMPAL CELLS

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Purpose: Long-term memory formation, expressed by cellular mechanisms including mGluR-LTD, requires local translation to alter specific synapses. RNA granules and particles both transport mRNAs to local sites in neurons, however, mechanisms of granule formation and translation regulation are poorly understood. The RNA-binding protein Staufen (Stau) is involved in mRNA transport, localization, and translational control. Staufen proteins, Stau1 and Stau2, are present in distinct ribonucleoprotein complexes and associate with different mRNAs. Stau1 is required for protein synthesis-dependent LTP (L-LTP), while there is also evidence suggesting that Stau2 is important in mGluR-LTD. We propose an underlying mechanism in determining these roles is the regulation of distinct RNA granules. **Methods:** The 3'UTRs of transported mRNAs (CaMKII - L-LTP, Map1b - mGluR-LTD, or Map2 - constitutive) were tagged with MS2 binding sites and detected by co-expression of MS2-mCherry in hippocampal cells. The distribution of mCherry puncta was measured in cells where Stau1 or Stau2 was knocked down using siRNA probes (n=3-4) and in response to an mGluR-LTD stimulus (DHPG) (n=3-4). Colocalization of Stau2-GFP, the ribosomal marker P0, and mCherry was also determined in response to DHPG (n=3-4). **Results:** Dendritic localization of Map1b mRNA was reduced in Stau2, but not Stau1, knockdowns. Moreover, DHPG stimulation induces Map1b, but not Map2, mRNA dissociation from RNA granules containing Stau2 and P0. This dissociation was dependent on Stau2. **Conclusion:** We show that Stau2:Map1b mRNA granules are specifically dissociated in response to mGluR-LTD stimuli. The knockdown results suggest a role for Stau2 in the generation and regulation of Map1b mRNA granules.

POS-WED-023

CHOLINESTERASE INHIBITION POTENTIATES POSTSYNAPTIC RECEPTOR LOSS IN A MOUSE MODEL OF ANTI-MUSK MYASTHENIA GRAVIS

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Purpose: Myasthenia gravis (MG) is the most common autoimmune disease of the neuromuscular junction (NMJ). MG is primarily caused by autoantibodies against acetylcholine receptors (AChR-MG). However, autoantibodies against the muscle-specific receptor tyrosine kinase (MuSK) are now thought to be responsible for many of the remaining cases (MuSK-MG). In mouse models, binding of these autoantibodies seems to cause disassembly of the NMJ, leading to characteristic muscle weakness. Pyridostigmine, a reversible acetylcholinesterase (AChE) inhibitor, is routinely used in the treatment of MG. It enhances the actions of synaptic-cleft acetylcholine upon residual AChRs. However, non-responsiveness and/or poor tolerability of AChE-inhibitor therapy has been noted in several clinical reports on patients with MuSK-MG. **Method:** We used the passive transfer model of MG by daily injecting IgG from anti-MuSK-positive MG patients into 6-week female C57Bl6J mice. Pyridostigmine was delivered by osmotic mini-pumps from day 8. At day 15, muscles were assessed for structural analysis of the NMJ by confocal fluorescent imaging. **Results:** Injection of MuSK-IgG into mice led to a fragmentation of the postsynaptic AChR clusters. This fragmentation impaired the degree to which the anti-synaptophysin-stained nerve terminal overlapped with its associated postsynaptic AChR cluster. However, in mice treated with pyridostigmine, NMJs (particularly in the diaphragm) showed significantly exacerbated loss of these co-localised AChR-clusters under the remaining nerve terminals. Pyridostigmine *per se* caused no impairment of postsynaptic AChR clusters or colocalisation. **Conclusion:** These histological findings suggest that pyridostigmine interacts with pathogenic mechanisms of anti-MuSK MG to further impair postsynaptic AChR clustering at the NMJ.

POS-WED-024

LOW-DOSE GYKI-52466 PRECONDITIONING IN A RAT MODEL OF BRAIN INJURY

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Purpose: Experimental preconditioning provides beneficial outcomes in conditions such as cardiac surgery, brain surgery and stroke. Recently, we showed that low-dose GYKI-52466 preconditioning imparts significant protection against kainic acid induced seizures. Hence, the present study evaluated the preconditioning effects of low-dose GYKI-52466 using a hypoxic-ischemic (HI) model of brain injury in adult rats. **Methods:** Two groups (n=11 each) of PND26 male Sprague Dawley rats were administered saline or GYKI-52466 (3 mg/kg, s.c.) 90 minutes before left common carotid artery ligation, and allowed to recover for 2 hrs prior to placement in a hypoxia chamber (8% O_2/92% N_2; 33±1°C) for 1 hr. Seizure activity was scored during HI, and sensorimotor tests were performed before surgery and at 1, 7 and 14 days post-HI. On day 14, brains were harvested, fixed and sectioned for assessment of infarct size and ventricular enlargement using ImageJ software. All observations were conducted blind to treatment. **Results:** Low-dose GYKI-52466 preconditioning significantly ($p<0.05$) reduced infarct volume (10.1 vs 174.1 mm^3), ventricular enlargement (4.0 vs 44.8 mm^3), seizures and gross morphologic indices of brain damage, and reduced foot-faults and postural reflex scores on all observation days. Grip strength was significantly higher in the GYKI group on day 14 (7.9 vs 3.9 min), and in a rearing test, saline controls exhibited significantly greater asymmetry in paw use during weight support relative to GYKI-treated animals. **Conclusion:** The present results indicate that GYKI-52466 preconditioning can provide protection against HI-induced brain injury at a dose approximately 10-fold lower than doses known to produce toxicity, and add support to the idea that low-dose GYKI-52466 neuroprotection involves metabotropic rather than classical ionotropic mechanisms.

POS-WED-025

SOURCES OF SPINE CALCIUM IN THE BASOLATERAL AMYGDALA

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Purpose: Emotional associative learning is thought to be mediated by long-term potentiation (LTP) of glutamatergic synaptic transmission in the basolateral amygdala (BLA). At nearly all synapses, LTP requires a rise in postsynaptic calcium. There are several mechanisms to raise postsynaptic calcium including calcium entry via ionotropic glutamate receptors and voltage-dependent calcium channels along with release of calcium from intracellular stores. Pharmacological dissection of the ion channel and receptor subtypes that contribute to postsynaptic calcium entry is confounded by the action of these pharmacological agents on transmitter release. Here we have dissected the calcium response to photoreleased glutamate at individual spines, avoiding presynaptic complications. **Methods:** Rats (21-28 d) were anesthetized with isoflurane, decapitated, and slices were prepared. Whole-cell patch-clamp recordings and high-speed calcium fluorescence images were made from BLA projection neurons in rat brain slices. Synapses were stimulated either electrically or via two-photon photolysis of caged glutamate. **Results:** Electrical synaptic stimulation evoked a spine specific in calcium that was completely abolished by application of the NMDA receptor antagonist APV (n = 5). Using photolytic uncaging of glutamate we were able to evoke a synaptic potential and spine calcium response that was comparable to the electrically evoked response. Preliminary data indicate that the calcium rise evoked by glutamate uncaging is largely mediated by NMDA receptor activation. Ongoing experiments are examining the contribution of various receptor subtypes and ion-channels to the postsynaptic calcium response. **Conclusion:** The postsynaptic calcium response to brief synaptic stimulation in BLA neurons involves NMDA receptor activation.

POS-WED-026

THE EFFECTS OF PLANT-DERIVED CHEMICALS ON 5-HYDROXYTRYPTAMINE AND ACETYLCHOLINE EVOKED CONTRACTION OF SMOOTH MUSCLE

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The plant-derived chemicals 1,8-cineole, lavender, linalool and *trans*-2-hexenal are increasingly being used in household products and complementary medicine. However, the effects of these plant-derived chemicals on 5-hydroxytryptamine and acetylcholine evoked contraction of smooth muscle have not been well characterised. **Purpose:** The present study examined the effects of 1,8-cineole, lavender, linalool and *trans*-2-hexenal on 5-hydroxytryptamine and acetylcholine evoked contraction of rat ilea. **Methods:** Sections of ilea from male Wistar rats were mounted in organ baths. Contraction was evoked by 5-hydroxytryptamine and acetylcholine in the presence of 1,8-cineole (n=6), lavender (n=6), linalool (n=6), *trans*-2-hexenal (n=6) or no plant-derived chemical (control) (n=12). Concentration-response curves were generated by adding increasing concentrations of 5-hydroxytryptamine and acetylcholine ranging from 1×10^{-9} M to 3×10^{-5} M for 5-hydroxytryptamine and 1×10^{-9} M to 3×10^{-4} M for acetylcholine. All plant-derived chemicals were examined at a concentration of 0.03% (vol/vol). **Results:** Lavender, linalool and *trans*-2-hexenal induced significant reductions ($p<0.05$) in 5-hydroxytryptamine evoked contraction from 3×10^{-7} M to 3×10^{-5} M and in acetylcholine evoked contraction from 1×10^{-7} M to 3×10^{-4} M. 1,8-cineole induced a significant reduction ($p<0.05$) in 5-hydroxytryptamine evoked contraction from 1×10^{-6} M to 3×10^{-5} M and in acetylcholine evoked contraction from 3×10^{-7} M to 3×10^{-4} M. **Conclusion:** Our present findings indicate that lavender, linalool, *trans*-2-hexenal and to a lesser extent 1,8-cineole reduce both 5-hydroxytryptamine and acetylcholine evoked contraction of smooth muscle by a non-receptor specific mechanism of action.

POS-WED-027

OPTOGENETICS: PROBING THE FUNCTION OF DISTAL DENDRITES

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Purpose: Understanding how dendrites process the thousands of synaptic inputs they receive is vital to understanding how the brain codes information. The advent of optogenetic methods provides a potentially powerful new tool for studying this issue. Here we investigate the impact of dendritic depolarisations on action potential firing in cortical neurons using optogenetics. This technique allows selective activation with high spatiotemporal resolution, which is difficult to achieve with existing tools. **Methods:** Brain slices were prepared from transgenic mice expressing channelrodopsin-2 (ChR2) under the control of the Thy1 promoter. Somatic whole-cell recordings were made from cortical layer 2/3 and 5 pyramidal neurons, as well as interneurons, identified by cell morphology, firing pattern and input resistance. Photo-stimulation of ChR2 was achieved with 470 nm light pulses targeted to the soma or specific dendritic regions. **Results:** We first assessed which neurons expressed ChR2. Photo-stimulation reliably evoked action potentials in all layer 5 pyramidal neurons examined (n=11). In contrast, photo-stimulation evoked action potentials in only a sub-set of layer 2/3 pyramidal neurons (4 out of 12 cells) and interneurons (3 out of 4 cells), indicating low and heterogeneous ChR2 expression in these cell types. Responses in layer 5 pyramidal neurons where not blocked by the AMPA-receptor antagonist DNQX (20 μM), indicating they are generated by direct postsynaptic depolarisation rather than via glutamate release from presynaptic neurons. Targeting photo-stimulation to progressively more distal areas of the dendritic tree of layer 5 pyramidal neurons revealed a steady decrease in the somatic depolarisation (n=4), consistent with passive attenuation. Despite marked attenuation, photo-stimulation of the apical tuft generated a reliable response at the soma. **Conclusion:** Optogenetic methods offer a powerful new approach for studying dendritic integration.

POS-WED-028

SELECTIVE LOSS OF AMPA RECEPTOR SUBUNITS AT CEREBELLAR SYNAPSES IN THE STARGAZER MOUSE

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Purpose. AMPA receptor subunits (GluA1-4) are trafficked to membrane synaptic sites by transmembrane AMPA receptor regulatory proteins (TARPs). In the stargazer mutant mouse, expression of TARP-γ2 (stargazin) is severely reduced, resulting in cerebellar ataxia and absence epilepsy. Stargazer granule cells (GCs) have a complete loss of AMPARs due to γ2 being their main TARP, hence mossy fibre (MF)-GC synapses are silent. The aim of the current study was to investigate how loss of stargazin affects the expression levels of AMPAR subunits at synapses between GC parallel fibres (PF) and other cerebellar neurons. **Methods.** Cerebella from male litter-pairs of stargazer and control mice (n=6) were prepared and analyzed by post-embedding immunogold-microscopy. Expression levels of GluA2/3 and GluA4 were evaluated by measuring relative density of immunogold at PF-Purkinje cell (PF-PC) and PF-interneuron (PF-In) synapses respectively. In total, 100 immunogold-labelled synapses were analyzed in each pair of stargazer and control littermates. **Results.** GluA2/3 and GluA4 expression was significantly reduced throughout the stargazer cerebellar cortex. At immunogold-labelled PF-PC synapses, GluA2/3 levels were reduced by 52% ($p<0.001$) in stargazer compared to controls. At PF-In synapses GluA4 levels were decreased by 29% ($p<0.05$) in stargazers. **Conclusion.** GluA2/3 subunits are significantly reduced at PF-PC synapses in stargazers. Although PCs express stargazin, they also express other TARPs (γ3 and γ7), which may compensate in trafficking GluA2/3 to PC dendritic membrane and explain why these synapses are not silent like the MF-GC synapses. Likewise, only partial loss of GluA4 at PF-In synapses in stargazers may be explained by TARP compensation. These data suggest that the ataxic phenotype in stargazers is primarily due to absence of AMPARs at cerebellar MF-GC synapses.

POS-WED-029

MODULATION OF THE DOPAMINE TRANSPORTER BY NOVEL KAPPA-OPIOID RECEPTOR AGONISTS

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Purpose: Drug addiction is characterised by compulsive drug seeking and taking even when the adverse consequences are known. The dopamine transporter (DAT) is involved in addiction and the kappa-opioid receptor (KOPr) modulates DAT function. Therefore the design of novel KOPr agonists may lead to potential anti-addiction therapies. This study investigated the effect of the novel KOPr agonists DS1 and DS3 on DAT function in a cell model. **Methods:** Using live cell confocal microscopy the uptake of the fluorescent DAT substrate ASP^+ was used to measure changes in DAT function in cells transiently transfected with YFP-hDAT and myc-rKOPr. Total internal reflection fluorescence microscopy and cell surface biotinylation were used to investigate the effect of these compounds on cell surface expression of YFP-hDAT. In addition, western blotting was used to establish whether these compounds function through the ERK1/2 pathway. **Results:** ASP^+ uptake showed a dose dependent increase in DAT function with both DS1 and DS3 ($p<0.05$-0.001) which was nor-binaltorphimine and pertussis toxin sensitive ($p<0.001$). There was an increase in activated ERK1/2 after compound addition ($p<0.05$), and inhibition of ERK1/2 attenuated the increase in function ($p<0.001$). Treatment with these agonists for 30 min did not produce a change in cell surface expression of YFP-hDAT (n=8-12). **Conclusions:** The novel KOPr agonists DS1 and DS3 dose dependently increased DAT function in an ERK1/2 dependent manner, without changing cell surface expression of YFP-hDAT. This data will help to understand the mechanism of action of these novel compounds and may aid in developing new therapeutic compounds for treating drug addiction.

POS-WED-030

INTERACTIONS BETWEEN THE TRIMERISATION AND TRANSPORT DOMAINS OF THE EAAT/ASCT FAMILY OF TRANSPORTERS INFLUENCES THEIR CHANNEL PROPERTIES

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Human Excitatory Amino Acid Transporters (EAATs 1-5) are responsible for the synaptic clearance of extracellular glutamate and play a critical role in preventing excitotoxicity. Another member of transporters that belong to the same family but catalyze electroneutral exchange of Alanine, Serine, and Cysteine is ASCT1. ASCT1 shares ~40% homology with the EAATs, with high conservation of regions implicated in the transport process. Binding of the substrate and sodium ions to ASCT1 or EAAT1 activates an uncoupled anion conductance, which follows a chaotropic selectivity sequence of $Cl^- < Br^- < I^- < NO3^-$. These transporters form trimeric complexes with each subunit capable of transporting substrates and also incorporating a Cl^- channel. Transmembrane (TM) domains that undergo substantial movement during the transport process include TM3, TM6, TM7, TM8, along with helical hairpin loops (HP) HP1 and HP2. TM1, TM2, TM4, and TM5 form the trimerisation complex and stabilize the protein in the membrane. To examine the significance of the interactions between these two domains, an EAAT1/ASCT1 chimera (E1A1C) was constructed containing an EAAT1 trimerisation domain and an ASCT1 transport domain. The transporters were expressed in *Xenopus laevis* oocytes and substrate-activated currents were measured using the two electrode voltage clamp technique. The chimera exhibited exchanger characteristics similar to ASCT1. The EC_{50} for L-ser transport by E1A1C was 34±4 μM and 43±10 μM for ASCT1. The chimera exhibited altered anion permeability. Cl^- and Br^- permeate ASCT1, but for the chimera the permeation of these anions is greatly reduced. These results suggest that interactions between the trimerisation and transport domain influence channel properties.

POS-WED-031

THE INTERCALATED CELL MASSES OF THE AMYGDALA – NOT ONLY ONE POPULATION?

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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. We studied the electrophysiological synaptic properties of ITCs and found that these cells can express either Ca2+ impermeable AMPA receptors containing the GluR2 subunit (n=40) with linear IVs, or express inwardly rectifying, Ca2+ permeable AMPA receptors (n=15), suggesting that there are two distinct popultations of interneurons. To confirm our results we used 200 μM NASPM, a specific GluR2 lacking AMPAR antagonist. Intercalated cells with linear IV could not be blocked by NASPM, whereas for AMPA receptors with small RI we observed up to 50 % reduction in the EPSC (n=5). This could also be due to the expression of a variety of different AMPAR subunit combinations at different synapses of the same cell, but stimulating in different regions of the BLA seems to activate the same type of receptor. Since action potential backpropagation can also elevate intrinsic Ca2+ levels, we performed Ca2+ imaging experiments. These results show that a single action potential only propagates a maximal distance of 50 μm away from the soma (n=6), whereas a train of 4 action potentials at 200 Hz leads to a backpropagation of about 90 μm away from the soma (n=6). Our result suggests there are two distinct populations of ITC neurons. We are currently investigating their functional role.

POS-WED-032

KIDNEY CHLORIDE REGULATION IN THE MOUSE CNS

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Purpose: Intracellular $[Cl^-]$ in the CNS has previously thought to be only regulated by the cation cotransporters NKCC1 and KCC2/3/4. NKCC1 was believed to be the only Cl^- co-transporter with an inwardly directed flow of Cl^- in the CNS, while KCC (primarily KCC2, but also KCC3/4), whose expression begins shortly after birth in the mouse, maintains a low $[Cl^-]$ (<10 mM) to produce a hyperpolarizing Cl^- conductance essential for inhibitory GABA synapses. Previous studies have indicated that there may be other transporters that may also regulate chloride with the CNS. In this study we examined the expression of KCC2, NKCC1 and NCC1, which like NKCC1 pumps Cl^- into cells along the Na+ gradient but has thought to only be expressed in the kidney. **Methods:** BALBC mice aged between P1 to P28 were euthanised using an overdose of sodium pentobarbital 100 mg/kg and tissues were either collected unfixed or perfusion-fixed with 4% paraformaldehyde. Brains, olfactory epithelium, retinal and kidney tissues were examined using immunohistochemistry, western blots and RT-PCR to determine the expression and localization of transporters NKCC1, KCC2 and NCC1. **Results:** NCC1 and NKCC1 were expressed in olfactory sensory neurons and the glomeruli of the olfactory bulb at all ages examined (n=30). NCC1 was also expressed in neurons in the retina, hippocampus and Bergmann glia within the cerebellum. RT-PCR and western blot experiments confirm that NCC1 was expressed at low levels in the CNS, which is likely to explain why previous attempts failed to locate it in these tissues. **Conclusion:** We show for the first time that NCC1 is expressed not only in the kidney but also in variety of neurons and glial cells within the mouse CNS.

POS-WED-033

CIRCUITS UNDERLYING FEEDFORWARD AND FEEDBACK SYNAPTIC INHIBITION IN THE OLFACTORY CORTEX

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The piriform (primary olfactory) cortex (PC) is a three-layered paleocortex that is used increasingly as a model system for studying cortical sensory processing. Little is known about inhibitory synaptic circuits in the PC. We recently identified in the PC five major classes of GABA-releasing interneurons (Suzuki & Bekkers, *Cerebral Cortex*, in press). **Purpose:** Our aim was to determine how our five types of interneurons are wired into microcircuits in the PC. **Methods:** Experiments used 300 µm-thick slices from the anterior PC of 18-25 d-old GAD67-GFP mice, in which GABAergic neurons express GFP. Whole-cell recordings were made at 33 ± 1 ºC. **Results:** We first focused on feedforward inhibition, provided by interneurons in the input layer (Ia). With excitatory neurotransmission blocked, stimulation of layer Ia produced both fast- and slow-rising monosynaptic inhibitory postsynaptic currents (IPSCs) in principal neurons in layer II. Pair recordings (n=10) showed that slow-rising IPSCs were produced by layer Ia neurogliaform cells, fast-rising IPSCs by horizontal cells. We next examined feedback inhibition. With excitatory neurotransmission intact, layer Ia stimulation produced additional polysynaptic inhibition of principal neurons. We traced the sources of this inhibition by making tissue cuts, measuring synaptic latencies (n=24), and making pair recordings (n=17) from principal neurons and different types of interneurons in deeper layers. These experiments identified a powerful trisynaptic feedback inhibitory circuit involving fast-spiking layer III multipolar interneurons. **Conclusions:** Principal cells in the piriform cortex receive two kinetically-distinct types of feedforward synaptic inhibition provided by neurogliaform and horizontal cells in the input layer. Principal cells also receive at least one kind of feedback inhibition, provided by fast-spiking multipolar cells in deep layers.

POS-WED-034

LONG-TERM POTENTIATION ACTIVATES LATENT PRECURSOR CELLS IN THE MOUSE DENTATE GYRUS

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Purpose: The recent discovery of a large latent population of hippocampal precursor cells in the dentate gyrus of adult mice led us to investigate whether activation of this population is regulated by synaptic activity. **Methods:** Mice were unilaterally implanted for acute electrophysiology, in which perforant path-evoked synaptic potentials were recorded from the dentate gyrus region of the hippocampus. One of a number of tetanus protocols was delivered, after which the mice recovered for 48 or 96 hours before they were processed for the neurosphere assay or bromodeoxyuridine injection, respectively. **Results:** Long-term potentiation (LTP) induced by high-frequency stimulation of the perforant pathway *in vivo* produced a greater than 5-fold increase in the number of neurospheres cultured from the stimulated hippocampus, compared to the non-stimulated hippocampus ($n = 8$; $p < 0.01$). Furthermore, LTP generated a significantly greater number of bromodeoxyuridine/doublecortin double-positive cells ($n = 5$; $p < 0.05$). No increase in proliferation or neurogenesis was observed when the stimulation protocol failed to induce LTP. **Conclusion:** These results show that LTP can activate latent neuronal precursor cells in the dentate gyrus, thereby providing a direct mechanism for regulating synaptically driven neurogenesis. These findings may help explain how environmental signals can affect neurogenesis.

POS-WED-035

Ca^{2+}-DEPENDENT TETHERING OF SECRETORY GRANULES TO F-ACTIN IS MEDIATED BY MYOSIN VI IN NEUROSECRETORY CELLS

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Neuroendocrine secretory granules (SGs) function as storage sites of neuropeptides and hormones that are released upon stimulation. Prior to undergoing exocytic fusion with the plasma membrane, SGs undergo maturation and priming, a process dependent on Ca^{2+} and ATP during which SGs acquire the competence to fuse. The molecular mechanism(s) underpinning ATP-dependent priming is still unclear and may involved Ca^{2+}-dependent recruitment of cytosolic proteins linking SGs with the F-actin network to initiate their translocation to the plasma membrane. **Purpose:** Identify cytosolic proteins interacting with SGs in a Ca^{2+}-dependent manner and elucidate their role in neuroexocytosis. **Methods:** We used purified SGs to pull down Ca^{2+}-dependent cytosolic interactors and identified Myosin VI (MyoVI) by mass spectrometry. Confocal and TIRF microscopy were used to investigate the effect of expressing GFP-MyoVI and mutants on SG subcellular distribution and spatio-temporal behavior in PC12 cells. **Results:** Western blotting confirmed that MyoVI is recruited to SGs in a Ca^{2+}-dependent manner ($p<0.05$; $n=5$). GFP-MyoVI localized on round structures that co-localized with Lifeact-mRFP, an F-actin marker. SGs clearly clustered in the immediate vicinity of these GFP-MyoVI-positive structures. Expression of truncated MyoVI that is unable to bind F-actin (GFP-MyoVI-tail) significantly reduced the number of NPY-Cherry-positives SGS and caused a more cytosolic distribution of this intravesicular marker. Importantly, TIRF microscopy revealed that GFP-MyoVI-tail overexpression caused a reduction of SGs density near the plasma membrane and an increase in SGs average speed. **Conclusion:** Our results suggest a novel role for MyoVI in recruiting SGs to F-actin in a Ca^{2+}-dependent manner.

POS-WED-036

AN IMMUNOHISTOCHEMICAL STUDY OF GABAA AND GLYCINE RECEPTOR SUBUNITS IN THE NORMAL HUMAN HYPOGLOSSAL NUCLEUS

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Purpose: In order to gain a more detailed knowledge of the inhibitory system in motor regions of the human brainstem, the localisation of the α1 subunit of the GABAA receptor and Glycine receptors (GABAAR and GlyR) were investigated in the human hypoglossal nucleus. **Methods** Single and double immunohistochemical labelling techniques and high resolution light and confocal microscopy were used to localise GABAAR α1 subunit and GlyR subunits on hypoglossal motor neurons. **Results** The results showed that the majority of the typical large hypoglossal motor neurons showed punctate “synaptic-like” membrane labelling for both receptor types with a partial co-localisation of the punctate immunoreactivities for GlyR and GABAAR. Very few neurons had only one receptor subtype. Our results show that GABAAR subunits and GlyR subunits are mainly localised at different sites on the same neurons but with a variable co-localisation. **Conclusion:** This suggests that inhibition of human hypoglossal motor neurons occurs through a complex interaction of separate GABAA and Glycine receptor synaptic regions on individual neurons and also further suggests that both inhibitory receptors show a co-localisation at some synaptic regions.

POS-WED-037

IONOTROPIC GLUTAMATE RECEPTORS IN THE VENTRAL TEGMENTAL AREA MODULATE CORTICAL AND SUBCORTICAL INPUT TO THE MESOACCUMBENS DOPAMINE PATHWAY

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PURPOSE: Burst firing of dopamine neurons in the ventral tegmental area (VTA) results in a large transient dopamine release in the nucleus accumbens (NAc) that is hypothesised to be important in modulating goal-directed behaviour and signaling rewards. While glutamatergic projections from the prefrontal cortex (PFC) and the laterodorsal tegmentum (LDT) are critical in mediating such midbrain cell activity, research elucidating the underlying receptor types that regulate this afferent input is ongoing. Previous studies have implicated midbrain ionotropic glutamate receptors in regulating mesoaccumbens dopamine transmission. In this light, the present study aimed to investigate the role of these specific receptor subtypes in modulating PFC- and LDT-stimulated dopamine release in the NAc. METHODS: In vivo fixed potential amperometry (FPA), combined with carbon fibre recording electrodes in the NAc, was used in urethane (1.5 mg/kg, i.p) anaesthetised Sprague Dawley rats (n = 19). Dopamine oxidation current evoked by PFC or LDT electrical stimulation (15 pulses at 50 Hz and 800 µA every 30 seconds) was recorded to identify changes in dopamine release following midbrain NMDA or AMPA/kainate receptor inactivation. RESULTS: Micro-infusion of the AMPA/kainate antagonist CNQX (1 µg/µL) or the NMDA antagonist AP-5 (6 µg/µL) attenuated PFC-evoked dopamine release recorded in the NAc (CNQX $p < 0.05$; AP-5, $p < 0.05$), while CNQX and AP-5 similarly attenuated dopamine release elicited by stimulation of the LDT (CNQX, $p < 0.0005$; AP-5, $p < 0.05$). CONCLUSION: These findings suggest that AMPA/kainate and NMDA receptors in the midbrain modulate cortical and subcortical glutamatergic input to the mesoaccumbens dopamine pathway, potentially providing information concerning the pathophysiology of drug addiction and schizophrenia.

POS-WED-038

INTERACTION OF GABAC ANTAGONISTS WITH CONSTITUTIVELY ACTIVE RECEPTORS

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GABA is the major inhibitory neurotransmitter and activates three classes of GABA receptors; $GABA_A$, $GABA_B$ and $GABA_C$ receptors. This receptor classification is based on the subunit composition, gating and pharmacological properties. $GABA_A$ and $GABA_C$ receptors belong to the Cys-loop ion channel family and mediate fast synaptic inhibition when activated by GABA. The agonist and/or competitive antagonist binding site is within the N-terminal region and located at the interface of two subunits. Five discontinuous regions form the binding sites from residues located on loops A-E. Tyrosine 102 (Y102), located in loop D of *rho*1 and the corresponding mutated receptor Y102S has previously been shown to form constitutively activitive receptors and the antagonist 3-APMPA was shown to be an inverse agonist. **Purpose**: Investigate whether all $GABA_C$ antagonists are inverse agonists on this receptor. **Method**: Two-electrode voltage clamp using *Xenopus laevis* oocytes. **Results**: The activities of GABA and the $GABA_C$ antagonists were shifted to the right on this mutation compared to wildtype receptors. Furthermore, most $GABA_C$ antagonists exhibited partial/ or full inverse agonist effects with EC50 values: TPMPA 1234.25 ± 57.72 µM (n=4), (±)-3-*cis*-ACPBPA 488.32 ± 60.54 µM (n=5), 4-GBA 544.6 ± 3.20 µM (n=2) and SR-95813 66.73 ± 0.44 µM. The exception was 4-aminocyclopent-1-enecarboxamide which appears to be a neutral antagonist. **Conclusion**: The efficacy depends on the chemical features of the compounds. $GABA_C$ receptors have distinct agonist and/or competitive antagonist binding site from other classes of GABA receptors. This is the first study to demonstrate that the majority of $GABA_C$ antagonists are inverse agonists and some remain neutral antagonists.

POS-WED-039

COCAINE INCREASES EXCITATORY INPUTS ONTO OREXIN NEURONS

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Purpose: The hypothalamic neuropeptide orexin (ORX), has been implicated in the modulation of drug-seeking behaviour and relapse. However, the effect of drug exposure on the synaptic inputs onto orexin neurons remains unknown. Interestingly, orexin neurons have been shown to rapidly 'adapt' to adverse environmental conditions, e.g. food deprivation. This 'soft-wired' circuitry may make orexin neurons highly susceptible to modulation by drugs of abuse e.g. cocaine. Thus, we determined whether cocaine altered the synaptic drive to orexin neurons using both anatomical and electrophysiological approaches. **Methods:** Rats (n=29) received intraperitoneal cocaine injections (15mg/kg; once/day for 7 days) or saline and processed for either anatomical or functional studies. For anatomical studies, dual-immunohistochemical labeling for orexin and either vesicular glutamate transporter (VGLUT2), a marker of excitatory inputs, or vesicular GABA transporter (VGAT), a marker of inhibitory inputs, was carried out. For functional studies, patch-clamp recordings were made to characterize miniature excitatory synaptic currents (mEPSCs) in presumptive orexin neurons. **Results:** Cocaine selectively increased the apposition of VGLUT2 puncta (24.08 ± 0.72 vs 20.10 ± 0.71 – per neuron, cocaine v.s. saline respectively) but not VGAT puncta (22.77 ± 0.6 vs 20.67 ± 1.44) onto orexin neurons. Furthermore, cocaine increased the frequency of mEPSCs (21.90 ± 5.13 Hz vs 8.31 ± 1.95 Hz) without affecting amplitude, rise or decay times. **Conclusions:** Anatomical and electrophysiological data indicate that cocaine exposure increases excitatory synapse formation and enhances the activity of new/or existing excitatory synapses. This increased excitatory drive may ultimately overexcite midbrain dopamine neurons, a downstream target of orexin neurons. This study identifies a potential cellular mechanism that may help to explain orexin's involvement in addiction and relapse risk.

POS-WED-040

THE MODULATION OF SODIUM CONDUCTANCE BY PHENYTOIN IS MEDIATED BY SLOWER RATHER THAN FAST INACTIVATION PROCESSES, AND IS UNAFFECTED BY FAST INACTIVATION REMOVAL

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Introduction Phenytoin (PHT) reduces the peak amplitude of voltage-gated sodium currents. It has generally been assumed that this occurs through effects on fast inactivation, but other slower inactivation processes may be involved. To clarify this we studied the effects of 50 uM PHT on both fast and slow inactivation processes. Methods Hippocampal slices from 3-7 week old Wistar rats were enzymatically treated and isolated CA1 neurons (n=36) obtained by mechanical dissociation. Inward currents were recorded using whole-cell voltage-clamp with a CsF based pipette solution. Steady-state inactivation (hinf) was measured with different length conditioning pulses, and dynamic transitions between closed states measured with double pulse protocols with timescales over several orders of magnitude (1 to 10000 ms). Persistent current (I_{Nap}) was also measured, and in some experiments papain (0.5mg/ml) was applied cytoplasmically to remove fast inactivation. Results PHT hyperpolarized the midpoint of hinf curves, moreso with longer conditioning pulses (~-3, -5 and -12 mV with 50, 150 and 500 ms pulses respectively, n=7). Properties of biexponential macroscopic inactivation and I_{Nap} were unaffected by PHT. Transitions between closed and closed inactivated states were slowed by PHT, but predominantly affected longer components rather than fast interconversions attributable to fast inactivation. Entry into slow inactivation was accelerated and recovery slowed by PHT. PHT had no effect of recovery of INa from fast inactivation, and exerted proportionally the same reduction in INa amplitude after fast inactivation removal. The effect of PHT appeared unaffected by the removal of fast inactivation with papain. Conclusions PHT appears to modulate sodium channels mainly through slow or intermediate duration inactivation processes rather than fast inactivation as has been commonly assumed.

POS-WED-041

GLYCOCONJUGATES WHICH BIND THE LECTINS IB4 AND WFA ARE CO-LOCALISED ON NOCICEPTORS BUT EXHIBIT INVERSE LECTIN BINDING CAPACITY IN RESPONSE TO CHRONIC CONSTRICTION INJURY

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Purpose: Glycoconjugates govern neural processes including axon guidance and synapse formation and so their expression by primary afferents may correlate with sensory modality. Lectins exhibit strong affinities to glycoconjugates thereby allowing visualisation of neurons with particular functions. **Aim:** As *Bandeiraea simplicifolia* I-isolectin B4 (IB4) is now routinely used to visualise subpopulations of nociceptors in health and disease, we aimed to determine whether IB4+ nociceptors also expressed binding sites for *Wisteria floribunda* agglutinin lectin (WFA) on the basis that WFA has an affinity for another glycoconjugate also shown to be expressed by nociceptors. **Methods:** Experimental rats (n=12, 3/group) were anaesthetised and the left sciatic nerve subjected to CCI. Naive rats (n=3) served as uninjured controls. After 7d,14d,21d,28d post-CCI, rats were perfused and lumbar spinal cord and DRG removed. Tissue was processed for fluorescent lectin histochemistry to visualise binding sites for IB4 and WFA. **Results:** Examination of DRG revealed WFA has a binding affinity for IB4+ nociceptors, although WFA binding sites are associated with cytoplasmic granules rather than the plasma membrane and Golgi bodies to which IB4 binds. Whilst lamina II of uninjured spinal cord displayed classic IB4 binding, very little WFA binding was observed. Conversely, lamina II of spinal cords 7d,14d,21d,28d after CCI exhibited not only the expected decrease in IB4 binding but also displayed a concomitant increase in WFA binding, superimposed over IB4 loss. **Conclusion:** Glycoconjugates for IB4 and WFA co-localise on a population of nociceptors but are located on distinct structures. The decrease in IB4 and concomitant increase in WFA binding capacity may insinuate variation in the mechanisms by which each glycoconjugate is transported axonally, and their function after injury.

POS-WED-042

CHANGES IN THE VISUAL SYSTEM OF THE MARMOSET MONKEY FOLLOWING PERINATAL HYPOXIC-ISCHEMIA

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Purpose: To investigate the implications of sublethal hypoxic-ischemic brain injury (HIBI) on the neonatal nonhuman primate visual cortex. **Methods:** Marmoset monkey (*Callithrix jacchus*) neonates at 30-36 hours of age were placed in a perspex container through which flowed gas containing 5% oxygen: 95% nitrogen for 1 hour (*n*=5). The control sibling was placed in a container through which flowed room air. Following hypoxia or control conditions, BrdU (100mg/kg, ip) was administered twice daily on 4 consecutive occasions (days 2-4). At P90 animals were euthanased, the brain fixed and the visual thalamic and cortical nuclei examined immunohistologically. **Results:** The cohort that underwent hypoxia exhibited an increase in GFAP (astrocytic marker) positive cells, which were distributed randomly throughout all 6 cortical layers of the primary visual cortex (V1). In both control and hypoxic groups there was no discernible difference in the density of BrdU+ neurones. However, there was a decrease in nonphosphorylated neurofilament (pyramidal cell marker) labeling in layer 3 throughout V1. A decrease in calbindin (interneurone marker) expression was also evident throughout the infragranular layers of V1. **Conclusion:** Whilst acute neuronal proliferation isn't dramatically affected at time of hypoxic ischemia (HI), the normal proportion of neuronal phenotype is disrupted that is likely to alter normal physiology of the visual system. These findings yield important new insights into the effect of HI on the development of the visual cortex with implications for the development of potential strategies to improve outcomes following acute HI in children.

POS-WED-043

METALLOTHIONEIN I/II AND METALLOTHIONEIN III INDUCE GROWTH CONE CHEMOTAXIS VIA DISPARATE MECHANISMS

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Metallothioneins (MTs) are 'stress response' antioxidant proteins implicated in neuronal protection and regeneration in injury and disease. MTs have an immunomodulatory and chemotactic role in leucocytes; however their role in neurite chemotaxis is yet to be elucidated. In this study, using the well established growth cone turning assay, the acute response of actively navigating embryonic (E16-18) rat sensory neurons to a gradient of MTI/II or MTIII was measured in vitro. Neurites were attracted to MTI/II (+9.8°±1.7°, n=11, p=0.0001), and repulsed by MTIII (-13.8°±1.9°, n=14, p=0.0001). The mechanism for MT-mediated chemotaxis was examined. MTs associate with members of the lipoprotein receptor-related protein (LRP) receptor family, LRP-1 and LRP-2 (megalin). Immunocytostaining of growth cones established that LRP-1 and megalin are distributed appropriately within growth cones for involvement in chemical sensing. LRP-receptor inhibitor, RAP, abrogated the chemotactic effect of MTs, suggesting that MT chemotaxis occurs via binding LRP receptors. Specific receptor involvement was investigated, utilising siRNA knock-down of LRP-1 and megalin, which demonstrated that LRP-1 is necessary for MT-mediated chemotactic signal transduction. MT-mediated chemotaxis is dependent on calcium ion concentration: when neurons were depleted of extracellular calcium, chemoattraction was converted to chemorepulsion, and vice versa. Pharmacological inhibition of MEK1/MAPK, calcium/calmodulin-dependent kinase II and calcineurin suggest that MTI/II and MTIII signal via disparate, but interplaying, pathways. Neurite chemotaxis is a functionally important component of neuronal regeneration, as regrowth must be directed to the lesion site, so that appropriate sprouting and ultimately new connections can be re-formed. Understanding the mechanisms by which MTs elicit this response in neuronal growth cones has important implications for future therapeutic developments in neuropathies and neuronal injury.

POS-WED-044

UNDERSTANDING COCHLEAR INFLAMMATION PROCESSES: LONGITUDINAL CHANGES IN VASCULAR PERMEABILITY

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Although inflammation is considered a major contributor to the development of ear disease and hearing disorders, most of the information is derived from acute animal studies and little is known about the progression of dynamic inflammatory disease in the living inner ear. **Purpose:** To chronically study the intact inner ear we have developed acquisition methods for magnetic resonance imaging of the intact and damaged cochlea and have investigated the evolution of dynamic changes in vascular permeability in cochlear tissues associated with inflammation in the same animal. **Methods:** To induce cochlear inflammation guinea pigs (GPs, n=4) were sensitised by bacterial lipopolysaccharide (LPS,0.8mg/kg) followed 24 hours later by bilateral LPS intra-tympanic injection (30µl). Two animals were treated with saline as a control. Anaesthetised GPs were scanned 4, 7, 10 and 14 days after inducing inflammation using a 4.7T MRI system combined with the use of a contrast agent (Gadodiamide, 500mmol/L, Gd) injected intravenously (femoral vein 1.5mmol/kg). Animals were scanned at fixed 1.5min intervals post-injection over a maximum of 70min and the rate of Gd uptake into cochlear tissues estimated as an index of vascular permeability. The rate of signal enhancement (Gd uptake) increased (3.5-fold) in the inflamed cochlea on day 4 and decreased to normal by 14 days indicating a reversible increase in vascular permeability with inflammation. **Conclusion:** These results demonstrate quantitative reversible changes in cochlear vascular permeability with inflammation and demonstrate the feasibility of using MR imaging to investigate dynamic, chronic changes in cochlear vascular perfusion in an animal model. This study was approved by the University of Auckland Animal Ethics Committee.

POS-WED-045

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

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Purpose: Seahorse retinas contain a convexiclivate fovea characterized by a rod-free depression where inner retinal layers are thinned in the centre and thickened on the edges. Preliminary data has indicated that these distinct characteristics develop with age. The aim of this study was to analyse the changes in foveal morphology and reactive distance, a behavioural measure of visual resolution, during development. **Methods:** Three groups of tropical *H. taeniopterus* (HT) seahorses were analysed. Group 1 (n=5) was composed of juvenile fish - 6 cm in length, Group 2 (n=5) fish were 10 cm in length and Group 3 (n=5) fish were adults - 15 cm in length. Reactive distance was tested for each group. Eyes were processed for frozen sections or flat-mounted. Wholemounts were stained with propidium iodide and photoreceptor (PR) densities determined. **Results:** The reactive distance in the larger fish was generally the longest, meaning that the bigger fish were able to detect smaller prey at the same distance compared with the smaller fish. Morphologically, the depression of the pit became deeper and more pronounced in the larger fish. Overall foveal PR density in Group 1 fish was 174,000 cells/mm^2. This foveal PR density increased to 180,000 cells/mm^2 in Group 2 and 243,000 cells/mm^2 in Group 3. **Conclusion:** HT fovea development is similar to that of other foveate retinas in that there is a deepening excavation of the fovea and an increase in PR with age. This change in morphology is correlated with an increased visual function.

POS-WED-046

ASSOCIATION BETWEEN BASSOON AND SYNAPTIC RIBBONS IN DEVELOPING MOUSE COCHLEA

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PURPOSE: Hearing depends on reliable and temporally precise neurotransmission established by a presynaptic specialization termed synaptic ribbon in cochlear hair cells. The number of synaptic ribbons reduces in the hair cells after birth, which is estimated as one of underlying causes of excess hair cell synapse elimination during the development, but the mechanism of how these synaptic ribbons are removed remain elusive. We hypothesized that a protein called Bassoon, essential for anchoring ribbons to synaptic membrane, may play a role in loss of synaptic ribbons in developing cochlea. **METHODS**: The expression of Bassoon and synaptic ribbons were identified by performing immunohistochemistry in mouse cochleae of postnatal day 0 (P0), 3, 6 and 12 (n=3 for each age). **RESULTS**: Bassoon and synaptic ribbons were well co-localized and their expression levels reduced from P0 to P12 in inner hair cells (IHCs). At P6, a few synaptic ribbons without Bassoon were identified. In outer hair cells (OHCs), there was a significant decrease in Bassoon levels from P3 to P6. The ribbons that were not co-localized with Bassoon were observed within the cytoplasm instead of baso-lateral membrane of OHCs at P6. **CONCLUSION**: These results suggest that Bassoon may aid the detachment of synaptic ribbons in both IHCs and OHCs during the development.

POS-WED-047

MCP-1 IS MAINLY EXPRESSED BY IB4+ NOCICEPTORS IN THE EARLY STAGES OF NEUROPATHIC PAIN

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Purpose: MCP-1 is expressed by primary afferents and triggers microglial infiltration into the spinal cord in models of neuropathic pain. The IB4+ subpopulation of nociceptors also contribute to neuropathic pain and their ablation is associated with a decrease in MCP-1 expression after injury. **Aim:** As MCP-1 represents a link between neurons and glia, our investigation was aimed at analysing the timecourse over which MCP-1 is expressed by primary afferents after CCI and ascertaining the proportion of MCP-1 expressing afferents which are IB4+. **Methods:** Experimental rats (n=4/group) were anaesthetised and the left sciatic nerve subjected to CCI. Naive rats (n=4) had no surgery. After either 4h,8h,24h,3d,6d,9d,14d or 21d survival post-CCI, rats were perfused. L4 DRG were removed and immunohistochemically processed to visualise both MCP-1 immunoreactive (IR), and IB4+, neurons in an area of 0.5mm^2 from each DRG. **Results:** An increase was noted in the number of MCP-1-IR neurons in the DRG ipsilateral to CCI in all injured rats with statistically significant increases (compared to naives) noted from 8h post-CCI and continuing, but reducing over time, up to 21d post-CCI. The peak of MCP-1 expression (five-fold increase compared to naives) was noted at 24h post-CCI. Moreover, at 24h post-CCI, 80% of the MCP-1-IR neurons were of small diameter and were IB4+. In comparison, at 21d post-CCI a higher proportion of MCP-1-IR neurons were larger in diameter with only 45% being IB4+. **Conclusion:** The majority of MCP-1 expression at early stages after CCI occurs in IB4+ nociceptors, confirming their role in initial microglial infiltration. However, later microglial infiltration may be mediated by larger diameter afferents.

POS-WED-048

STRUCTURAL CHANGES DURING THE DEVELOPMENT OF THE MOUSE DORSAL COCHLEAR NUCLEUS

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The cochlear nucleus (CN) in the medulla is the first central auditory nucleus and is the obligatory termination of the auditory nerve. The CN consists of three major subdivisions: anterior ventral, posterior ventral and dorsal cochlear nucleus (DCN). The DCN is a layered structure which receives extensive auditory and non-auditory inputs. DCN has a high level of plasticity but little is known about its development and the differential role of these inputs in refining its circuitry. **Purpose:** This study investigated the organisational development of the mouse DCN (P0-P21). **Methods:** Lipophilic dyes were applied to different cochlear regions to identify the afferent projections to the DCN. Nissl staining and immunohistochemistry (MAP2 and synaptophysin) were used to provide more detail of the neuropil and synaptic organisation in the developing DCN. **Results:** This study shows that the tonotopic innervation of the DCN is established early in development (by P0), however, the DCN is poorly organised at this stage. There were dramatic increases in DCN volume and changes in the cellular organization from P3 and the three distinct layers of the mature DCN were identifiable by P6. This organisational development was accompanied by a substantial decrease in cell density (P3-P12) and changes in the morphology of dendrites and distribution of synapses. There was relatively little change in the organisation thereafter. **Conclusion:** This study demonstrates that the DCN assumes an adult-like organisation by P12 and provides a platform for further investigations of the development of the neuronal circuitry in the DCN. This project was approved by the University of Auckland Ethics Committee.

POS-WED-049

ADAPTIVE OPTICS IMAGING AND ANALYSIS OF MARMOSET CONE PHOTORECEPTORS

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Purpose: To improve knowledge of the neural basis underlying colour vision in primates, it is vital to understand how cone photoreceptors are arranged and connected to other retinal neurons. This information can be learned through multi-spectral in vivo imaging of the cone photoreceptor mosaic using adaptive optics (AO). The purpose was to verify basic correspondence between AO images of the photoreceptors and histology in the marmoset (Callithrix jacchus). **Methods:** A compact and portable AO flood-illuminated ophthalmoscope was constructed and attached to an electrophysiology-recording rig. It was designed for imaging marmoset eyes, with wide field of view (5.2° diameter) projecting onto a 4 MP CCD camera. Aberrations were measured with a Shack-Hartmann device and corrected with a Mirao 52d deformable mirror over a 3.2 mm pupil dilated with tropicamide. Retinal images were collected from a sufentanil-anaesthetized, paralyzed marmoset at 15 Hz using 5 ms pulses of red light (671 nm). The animal was euthenased with sodium pentobarbitone at the end of the experiment and perfused with 4% paraformaldehyde in 0.1M phosphate buffer for 10 min. Eyes were extracted and immersion-fixed in the same solution for 20-30 min. Retinas were dissected and prepared as whole-mounts. **Results:** Cone photoreceptors were resolved in the AO images over the central 30° of the horizontal meridian, up to and including those near the optic nerve head and within 0.15 mm of the foveal centre. Fourier analysis was used to determine cone density over the measured field; recovered density peaked at over 120,000 cones/mm^2 and showed a naso-temporal asymmetry. Results agreed well with previously published data from histology. **Conclusion:** AO retinal images of the marmoset cone photoreceptor mosaic have been achieved. Density of AO identified cones corresponds well to retinal histology in the same eye.

POS-WED-050

NEUROINFLAMMATION AFTER PERIPHERAL NERVE INJURY IN MICE

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Purpose: Partial sciatic nerve injury is a common model of neuropathic pain in rodents, and produces both mechanical and thermal pain hypersensitivity. Several types of immune cells have been implicated in the pathogenesis of neuropathic pain due to nerve injury; however, the timing of their appearance has not been fully elucidated. **Methods:** Using immunohistochemistry, we characterized the time course and magnitude of inflammatory cell infiltration and resident immune cell activation in the sciatic nerves, L3-5 dorsal root ganglia (DRGs) and spinal segments at different time points after partial ligation of the sciatic nerve (PSNL) in C57BL/6J mice (n=4 mice per group). **Results:** PSNL markedly decreased paw withdrawal threshold to mechanical stimuli and paw withdrawal latency to thermal stimuli in the injured side. We found a significant increase in the numbers of infiltrating neutrophils, macrophages, dendritic cells and lymphocytes in the injured sciatic nerve and ipsilateral DRGs in comparison to sham-operated controls, with different timeline of recruitment for each cell type. Expression of ATF3 in the cell bodies of DRG neurons indicated 30-40% neuronal damage. No neutrophils, dendritic cells, and lymphocytes were found in the spinal cord. However, a significant increase in the level of microglial and astrocyte activation was observed in the spinal dorsal horn and to a lesser extent in the ventral horn, peaking on days 7 and 14 after nerve injury. These changes corresponded with a significant increase in phosphorylated NR1 subunit of the NMDA receptor, and a significant decrease in terminals of IB4-positive non-peptidergic nociceptive fibers in the ipsilateral dorsal horn. **Conclusion:** Our findings suggest differential roles for peripheral and central neuroimmune interactions in the production of neuropathic pain.

POS-WED-051

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) EXPRESSION IN MüLLER CELLS AS AN ASSAY OF NEUROPROTECTION IN THE VERTEBRATE RETINA

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PURPOSE: The Müller cell is the principal macroglial cell of vertebrate retina, which performs functions of neuronal support, transmitter recycling and ionic buffering, over most of its thickness. Unlike star-shaped astrocytes, each Müller cell is radial in form, extending from the inner to the outer limiting membranes of the retina. Under retinal stress, for example by excess light, Müller cells upregulate glial fibrillary acidic protein (GFAP), an intermediate filament protein characteristic of astrocytes, and their radial morphology is observable by immunohistochemistry which provides an avenue to investigate neuroprotectants based on their degree of expression. **METHODS**: Two forms of neuroprotection for retinal photoreceptors, dietary saffron and photobiomodulation (PBM; low level irradiation with red infrared light), were used. Adolescent albino Sprague-Dawley rats were prefed with dietary saffron (TAS and L'AQ) or pre-treated with PBM (830nm) 3 minutes daily, over seven days and exposed 24 hours to bright continuous light. 24 hours post recovery, rats were culled, retinas removed, fixed and cryosectioned. Sections were stained for apoptotic cells (TUNEL), Muller cells expressing glial fibrillary acidic protein (GFAP), and neuronal nuclei (Bizbenzimide) and compared to age matched controls. **RESULTS**: The expression of GFAP in Müller cells varies with levels of damage suffered by the retina. GFAP+ results suggest a significant difference ($p<0.05$) in GFAP expression in age matched controls (n=4) and PBM 830nm (n=5) and will be presented with corresponding TUNEL+ data. **CONCLUSION**: Preliminary results suggest GFAP+ length of Müller cells provides a consistent estimate of damage suffered by the retina and may provide a quantitative assay of the degree of protection achieved.

POS-WED-052

PATCH-CLAMP RECORDINGS IN DISSOCIATED SPIRAL GANGLION NEURONS

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Purpose: Amongst the candidate ion channels thought to contribute to changes in auditory nerve activity following deafness are the hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels. The mixed-cation I_h current to which these channels give rise is implicated in setting resting membrane potential and influencing firing frequency. This project examines the activity of I_h in spiral ganglion neurons (SGNs) of both early-postnatal and adult animals. **Methods:** *In vitro* preparations of dissociated SGNs were obtained from untreated early-postnatal rats (P3-5; n=5) and normal-hearing adult guinea-pigs (P21 or older; n=18). Patch-clamp electrophysiological recordings were used to assess neural activity and measure the contribution of I_h. **Results:** In both SGN models, whole-cell recordings in current-clamp revealed an activity profile consistent with activation of a hyperpolarisation-activated I_h current. Rapid membrane hyperpolarisation was followed by a voltage- and time-dependent depolarising sag, and a rebound action potential on stimulus offset. In voltage-clamp, a slowly activating inward current was observed without evidence of inactivation. **Conclusion:** Dissociated SGN preparations from early-postnatal rats and adult guinea-pigs show evidence of hyperpolarisation-activated currents.

POS-WED-053

MECHANISM UNDERLYING DISTENSION-EVOKED PERISTALSIS IN GUINEA-PIG DISTAL COLON: IS THERE A ROLE FOR ENTEROCHROMAFFIN (EC) CELLS ?

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Purpose: It has been proposed that the initiation of colonic peristalsis, following distension, is due to release of 5-hydroxytryptamine (5-HT) from enterochromaffin (EC) cells in the mucosa. However, no direct evidence exists to support this hypothesis because real time recordings of 5-HT release have not been made during colonic peristalsis. The aim of this study was to determine whether 5-HT release from EC cells was required for distension-evoked colonic peristalsis in isolated guinea-pig distal colon. Methods: Real time amperometric recordings of 5-HT release combined with video imaging were made during peristalsis evoked by fluid infusion and by fecal pellets. Results: Amperometric recordings revealed a basal release of 5-HT from EC cells of 22μM (n=7), during peristalsis evoked by fecal pellet distension. However, removal of the mucosa and submucosal plexus abolished all release of 5-HT, but did not inhibit the initiation of peristalsis, nor prevent the propagation of fecal pellets along the colon. Maintained distension by pellets generated cyclical peristaltic waves, which also persisted following removal of the mucosa and submucosal plexus, but at reduced pacemaker frequency. Perfusion of exogenous 5-HT (10μM) through the lumen did not initiate peristalsis, nor alter the velocity of peristaltic waves evoked by constant fluid-distension, in either intact preparations, or preparations with mucosa and submucosal plexus removed (n=5). Conclusion: The sensory neurons and mechanoreceptors which initiate peristalsis lie in the myenteric plexus and/or muscularis externa, and do not require any release of 5-HT from EC cells, the submucosal plexus, or activation of sensory nerve endings in mucosa, as previously hypothesized.

POS-WED-054

CHARACTERISING REMODELLING EVENTS IN THE INNER RETINA OF AGED RD1 MICE

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Purpose: Retinitis pigmentosa (RP) refers to a family of inherited diseases that lead to blindness. Previous studies in animal models of RP have shown retinal changes in either early stages of RP when photoreceptors are dying or at end stages just prior to animal death. Our research focuses on characterising the remodelling of retinal neural circuits in mid to late stages of the disease when most intervention methods for blindness would be desired. **Methods**: We have used rd1 mice that carry a mutation in the β subunit of phosphodiesterase 6 leading to RP. Rd1 and control mice were aged to three groups (n=6 for each age and strain): 3-5months, 6-8months and >11months. Central and peripheral regions of retina were collected, embedded in resin and processed for post-embedding amino acid immunocytochemistry. **Results**: The inner retinal layers were thinner in the rd1 mouse compared with controls indicating a widespread loss of cells in these retinae. However, remodelling events and glial changes occurred in patches across the retina. The migration of neural processes was evident in the central retina at 6-8months and >11months and glial scar formation and thickening was more prevalent at >11months. There was a gradual progression of remodelling events from the central to peripheral retina as these rd1 animals aged. We propose that these events might be linked to the pattern of cone death in the retina. **Conclusion**: Intervention methods such as a retinal prosthesis would need to be used early in the disease process to take advantage of remaining normal circuitry across the retina.

POS-WED-055

IN VITRO CHARACTERISATION OF NEURO-UROTHELIAL INTERACTIONS IN A NOVEL COCULTURE SYSTEM

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Purpose: The urothelium forms the epithelial lining of the lower urinary tract and is suggested to function as a sensory transducer, which detects chemical, mechanical or thermal stimuli, and signals to nerve terminals and other cells in the bladder wall. To facilitate study of neuro-urothelial function we developed a novel protocol for coculturing dorsal root ganglion (DRG) sensory neurons and bladder urothelial cells. **Methods**: DRG and bladders dissected from anesthetised and decapitated adult female Sprague-Dawley rats (6-8 wks), were enzyme treated, and mechanically dissociated to obtain isolated sensory neurons and urothelial cells. These were plated together, or separately, on collagen-coated substrate and cultured in keratinocyte medium for 48-72h. **Results**: The comparison of monocultured and cocultured neurons detected increases in neurite initiation (from around 30% to 70%, n=4) and neurite length and complexity in the cocultures. This growth-promoting effect of urothelial cells occurred in peptidergic (CGRP-positive) and non-peptidergic sensory neurons (n=3). Inhibition of tyrosine kinase signalling with K252a (n=3) blocked the growth respose but inhibiting upstream NGF signalling with TrkA-Fc had no effect (n=2). Glial cell number was decreased in cocultures (n=3). FURA-2 calcium imaging detected agonist responses of urothelial cells to ATP (100μM, n>5), but not agonists of TRPV1 (capsaicin, 1μM), TRPV3 (farnesyl pyrophosphate, 1μM), TRPV4 (4alpha-PDD, 10μM) or TRPA1 (mustard oil, 100μM)(all n>3). In contrast, all agonists except farnesyl pyrophosphate could induce neuronal calcium responses in the same cocultures (all n>3). **Conclusions**: Our results suggest urothelial cells can stimulate growth of cocultured sensory neurons independently of NGF signalling. We also found urothelial cells could be activated by ATP, but could not confirm reports of activation by TRP agonists.

POS-WED-056

CYCLOOXYGENASE MEDIATES WHISKER EVOKED BOLD FMRI RESPONSES DIFFERENTIALLY IN SOMATOSENSORY STRUCTURES IN RAT

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Purpose: Blood oxygen level dependent (BOLD) fMRI relies on the hemodynamic response that accompanies changes in neural activity. Characterisation of the hemodynamic response function and the mediators linking neural activity and hemodynamics are therefore important for the interpretation of fMRI data. However, studies are typically conducted within a single brain region and inferences from such studies implicitly assume the neurovascular coupling relationship to be homogeneous across the brain. The present investigation therefore, uses high field fMRI in a rodent model to measure BOLD responses in several structures in the somatosensory pathway: brainstem, thalamus and cortex. **Method:** The whisker pad of urethane anaesthetised rats (N=6) was electrically stimulated (1.6mA, 40s) using a range of stimulation frequencies from 1-20Hz. Whole brain BOLD echo planar imaging imaging was performed using a birdcage coil within a 7T Bruker MR spectrometer. **Results:** BOLD fMRI responses in cortex were greatest in magnitude at lower stimulation frequencies. Responses elicited by midrange frequencies were greatest in magnitude in the thalamus while responses elicited by high frequencies were greatest in magnitude in the brainstem. Indomethacin, a non-specific inhibitor of cyclooxygenase, was administered intravenously (5mg/kg or 5mg/kg/hr) to examine its effects on whisker evoked BOLD responses in these different somatosensory structures. Following administration of indomethacin, stimulus evoked BOLD responses were attenuated differentially across the whisker to barrel pathway. The responses recorded in brainstem displayed the least amount of attenuation, with responses from thalamus and cortex showing much greater reductions. **Conclusion:** These data suggest that the cyclooxygenase pathway has greater involvement in linking neural activity to hemodynamics in the cortex and thalamus than the brainstem.

POS-WED-057

BRAIN DYNAMICS IN THE FLY AND THE BEE: TIMING AND VISUAL COMPETITION

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Purpose: How does the visual system process basic visual features, such as color or spatial location in a dynamic environment? In order to answer this question, we investigated brain dynamics during visual processing using electrophysiological techniques in honeybees and flies. **Methods:** Honeybees (*Apis mellifera*) and fruit flies (*Drosophila melanogaster*) were immobilized and multi-electrodes (Neuronexus Technologies) then inserted into specific brain regions to record brain activity. In *Drosophila*, 16 recording sites of the multi-electrode array spanned the entire brain of the fly, allowing us to record the local field potential (LFP) in multiple brain areas at once. In bees, we recorded up to 32 channels of brain activity in multiple brain regions, which included LFPs as well as spiking activity. The visual stimuli included flickering light emitting diode (LED) arrays or CRT screens at different locations around the bee or fly. **Results:** Recordings were performed from both bees (n=44) and flies (n=40)while presenting competing visual cues. When presenting competing visuals, which included competing flickering light stimuli pointed toward each eye of the bee or fly, the flicker frequency can be detected in the brain activity. One or the other flicker frequency dominated in the brain dynamics, with alternation between the dominant frequency occurring every few seconds. This alternation is significantly higher in central brain areas than periphery (n=10, ANOVA, $p<0.001$). **Conclusion:** Experiments so far indicate that alternation dynamics of brain activity in flies and bees occur in response to competing visual stimuli, but by further investigating how the brain can process dynamic visual cues, we can gain a better understanding how the visual system interfaces with the world around it.

POS-WED-058

CHRONIC SWELLING AND ABNORMAL MYELINATION DURING SECONDARY DEGENERATION OF THE OPTIC NERVE

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Purpose: To assess chronic changes in optic axons and myelin sheaths during secondary degeneration. **Methods:** The dorsal aspect of optic nerve (ON) in Piebald-Virol-Glaxo rat was transected, leaving central/ventral ON undamaged, but vulnerable to secondary degeneration. Transmission electron microscopy of central/ventral ON at 1 and 3 months was used to quantify secondary changes in axon diameter, myelin sheath thickness and g ratios. Densities of myelinated, unmyelinated and abnormally myelinated axons (categorised into 4 sub-populations according to myelin sheath morphology) were analysed. All data were compared to normal animals. **Results:** Cross sectional nerve area at the injury site increased at 3 months ($p\leq0.05$) and changes in axons and myelin sheaths were detected in central/ventral ON. Although myelin sheath thickness was unchanged, average axon diameter increased at 3 months ($p\leq0.05$), while g ratios increased at 1 and 3 months ($p\leq0.05$). Density of the total axon population decreased by 1 month ($p\leq0.05$), with a decrease in density of normally myelinated axons ($p\leq0.05$), reflecting our published reports of retinal ganglion cell death, but an increase in density of unmyelinated axons ($p\leq0.05$). Within the 4 sub-populations of abnormally myelinated axons there was: no change in lightly myelinated axons (1 and 3 months; $p>0.05$); an increase in axons with excessive myelination (1 month, $p\leq0.05$); and an increase in density of axons with partial and fully-decompacted myelin (3 months, $p\leq0.05$). **Conclusion:** Chronic increases in axon calibre and myelin sheath compaction defects are features of secondary degeneration, and may contribute to the reported loss of visual function following partial ON transection.

POS-WED-059

ROLE OF COMPLEMENT AND CHEMOKINE IMMUNE RESPONSE PATHWAYS IN A LIGHT-INDUCED DEGENERATIVE MODEL OF ATROPHIC AMD

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Purpose: To investigate the retinal expression and localization of inflammatory markers from chemokine and complement system pathways following degeneration induced by excessive light. **Methods:** SD rats were exposed to 1000lx of light for up to 24hrs. At specific time-points during (1, 3, 6, 12, 17, and 24hrs) and following (3 and 7 days) exposure, animals were euthanized and retinas processed. Photoreceptor apoptosis was assessed using TUNEL (n=5 per time point) and counts were made of monocytes immunolabeled with ED1 (n=4 per time point). Expression of chemokines and complement components were assessed by microarray analysis (n=3 per time point). Ccl2 and C3 were selected for spatiotemporal analysis by immunohistochemistry, and in situ hybridization (n=3 per time point). The One-way ANOVA, and Student's t-test were used for statistical analysis, with a P-value threshold of <0.05. **Results:** Exposure to bright light induced focal degeneration on the visual axis that was associated with significant up-regulation of complement and chemokines and with local aggregations of monocytes in the retina and choroid. Immunohistochemistry and in situ hybridization show that Ccl2 is expressed by Müller cells in the incipient lesion. C3 was expressed by infiltrating monocytes from the neural retina, in association with the expanding retinal lesion. **Conclusions:** Our data indicate that the retina contributes to the neuroinflammatory response following injury, by local expression of inflammatory mediators which attract C3-bearing monocytes. These findings have relevance to the underling pathogenesis of atrophic AMD.

POS-WED-060

THE EFFECT OF NOISE EXPOSURE ON SPIRAL GANGLION NEURONS IN $P2X_2$ KNOCKOUT MOUSE COCHLEA

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ATP-gated ion channels assembled from P2X2 receptor subunits are expressed by the cochlear spiral ganglion (SG) neurons. The $P2X_2$ knockout (KO) mice show a significant exacerbation of permanent threshold shift (PTS) after exposure to loud noise compared to wild type (WT) mice. **Purpose:** In this study, we examined the effect of noise exposure on the morphology of the SG neurons in both WT and $P2X_2$KO mouse cochleae. **Methods:** Age-matched (8-10 weeks) groups of $P2X_2$KO mice (n=4) and C57BL/6 WT control mice (n=5) were exposed to open-field traumatic noise (2 hours; 100 dBSPL, 8-16 kHz). The cochleae were fixed using 4% paraformaldehyde (PFA) in 0.1M phosphate buffer, decalcified in 8% EDTA, then embedded in epoxy and cut into thin sections. Cochlear sections were stained by H&E and scanned by Aperio ScanScope XT Slide Scanner. An image of the mid-turn SG region from each cochlear section was analyzed using Image Pro® Plus 6.2. The neuronal size, number and the total area of each SG section were measured. Statistical analysis (unpaired t-test) between the average sizes and the number of SG neurons/1000 μm^2 in $P2X_2$KO and WT was performed using Graphpad® Prism 4. **Results:** The $P2X_2$KO mice showed smaller sized mid-turn SG neurons compared to WT mice ($p=0.0084$). No significant difference in the number of neurons/1000 μm^2 was detected. The morphology of the organ of Corti, including the hair cells, was comparable in both $P2X_2$KO and WT mice. **Conclusion:** The enhanced PTS with the lack of $P2X_2$ receptor expression is associated with a significant reduction in the size of the spiral ganglion neurons in the cochlea.

POS-WED-061

CHARACTERISATION OF LPS-INDUCED COCHLEAR INFLAMMATION

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Infection of the middle ear (otitis media), a prevalent inflammatory disease among young children, is a common cause of hearing impairment. Inner ear inflammation is a serious complication of otitis media and is involved in the pathogenesis of the cochlear injury and hearing loss. This occurs by the spread of inflammatory agents from the middle ear into the inner ear through the round window membrane, a soft tissue barrier that separates the two compartments. **Purpose**: We aimed to investigate the cochlear inflammatory response by establishing and characterising an animal model of cochlear inflammation based on otitis media. **Methods**: Middle ear inflammation was created in outbred CD-1 mice (*n*=4) and Wistar rats (*n*=5) by systemically sensitising the animals to *E. coli* endotoxin lipopolysaccharide (LPS) and subsequently (24 hours) challenging the middle ear with the LPS endotoxin. Controls were treated in an identical manner but with sterile saline. Cochleae were examined 3 days later for the presence of inflammatory cells by performing immunohistochemistry for CD45, a cell surface marker of bone marrow derived leukocytes, and F4/80, a marker of activated macrophages and monocytes. **Results**: Following induction of LPS-induced middle ear inflammation, a large infiltration of CD45+ and F4/80+ inflammatory cells was observed within the cochlea. These were predominantly localised in the perilymphatic spaces as well as in the spiral ligament and spiral ganglion. **Conclusion**: The cochlear inflammatory response following LPS-induced otitis media involves the recruitment of inflammatory cells. Further studies are required to obtain a better understanding of the molecular processes underlying cochlear inflammation, which may potentially lead to the development of novel therapeutic interventions for hearing loss associated with cochlear inflammation. This study was approved by the University of Auckland Animal Ethics Committee.

POS-WED-062

TASTE IDENTIFICATION THRESHOLDS IN ADULTS WITH AUTISM SPECTRUM CONDITIONS

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Purpose: In addition to social and communication problems, sensory issues have been widely reported in Autism Spectrum Conditions (ASC). Taste perception is one of the least investigated senses in ASC. The current study thus explored taste identification thresholds in adults with ASC. **Methods:** 23 adults with ASC (12 males, 11 females) were compared to 26 IQ- and age-matched control participants with no history of psychiatric conditions (14 males, 13 females). A psychophysical chemical taste test, the Taste strips (Burghart, Medizintechnik, Germany), was used to measure taste identification threshold overall, as well as bitter, sour, sweet and salty tastes. **Results**: Multivariate tests revealed a significant effect of group on taste identification ($F(5,46)=2.78$, $p=.02$). Separate univariate ANOVAs showed lower taste identification accuracy overall in the ASC group ($F(1)= 10.20$, $p=.003$), as well as for bitter taste ($F(1)=9.27$, $p=0.004$), sour tastes ($F(1)=5.71$, $p=.02$) and sweet tastes ($F(1)=4.03$, $p=0.05$). However, salty taste thresholds did not differ between the groups ($F(1)=2.98$. $p=.09$). **Conclusion**: Adults with ASC were less accurate in identifying tastes taste overall, particularly in relation to bitter, sour and sweet tastes. However the groups did not significantly differ in identifying salty tastes. We suggest that this difference is unlikely to reflect any basic sensory difference in ASC, but instead might reflect task difficulties such as verbal labelling of tastes.

POS-WED-063

ADAPTIVE REGULATION OF THE ENDOCOCHLEAR POTENTIAL IN THE MAMMALIAN COCHLEA

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Transduction process in cochlear hair cells relies on the electrochemical integrity of the endolymphatic space. The key parameter is the positive endolymph potential (endocochlear potential, EP) which is tightly regulated to maintain cochlear sensitivity. The homeostatic regulation of EP, particularly during sound exposure is not well understood. **Purpose:** This study describes the interplay between EP and conductance's in the endolymphatic compartment (cochlear partition resistance, CoPR) with changes in endolymph volume and loud sound exposure. **Methods:** Experiments were undertaken in mice (n=6 each group). EP was measured from cochlear endolymph using micropipettes filled with artificial endolymph and CoPR was measured by passing square current pulses (1μA) and measuring voltage changes. ATP was introduced into endolymph to activate a shunt conductance via $P2X_2$ receptors in control and noise exposed (100dB, 48hrs) animals and furosemide (10-200mg/kg ip) was used to inhibit the Na^+-K^+-Cl^- exchange pump on marginal cells, responsible for generation of EP. **Results:** Injection of ATP into endolymph caused a fall in EP and reduced CoPR, followed by compensatory recovery of EP without an initial change in resistance. CoPR recovers after noise exposure, but EP remains low after the initial fall causing a hysteresis effect. Increasing endolymph volume decreased EP with a concomitant increase in resistance. Furosemide caused a decrease in EP with a concomitant increase in resistance. **Conclusion:** Changes in CoPR in the face of a falling EP probably reflect a compensatory adaptive mechanism to restore the EP. This indicates tight coupling between EP and resistive pathways in cochlear tissues to maintain hearing sensitivity. This may be affected by noise injury. *Approved by the University of Auckland Animal Ethics Committee.*

POS-WED-064

SPATIAL DISTRIBUTION OF APOPTOTIC MARKERS IN RETINAL DEGENERATION

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Purpose: To investigate the spatial display of cell death in retinal degeneration using two distinct models of retinal disease, a light damage model of photoreceptors death and an ischaemia/reperfusion rat model of inner retinal cell death. **Methods:** Photoreceptor cell death was induced by exposing Sprague-Dawley (SD) rats to intense light levels (2700 lux) for 24 hours followed by 6 hours recovery. Inner retinal cell death was induced by increasing intraocular pressure (120mmHg for 1.5 hours) followed by 24 hours reperfusion. The animals (n=6 per condition) were killed and the retina was dissected, separated from the rest of the eye tissue and processed for immunocytochemistry. The antibodies used were detecting apoptotic markers (Bax, caspase 3, caspase 9, cytochrome c). The authophagic pathway was investigated using lamp-1. We employed bcl-2 to detect anti-apoptotic function in both retinal models. **Results:** In both models there was expression of Bax in the inner nuclear layer and selective expression in cells in the choroid in the light damaged retina. Cleaved caspase-3 was observed in subpopulation of cells in the light damaged photoreceptor layer, and in some cells in the inner retina of the ischaemic/reperfusion model. Caspase 9 was observed in the retinal pigmented epithelium (RPE) of the light damaged retina and was absent in the ischaemic retina. There was no cytochrome c labelling in the ischaemic model although it was expressed in the choroid in the light damaged retina. Lamp-1 and bcl-2 expression was confined to the RPE and choroid in the light damaged retina where there was copious TUNEL labelling in the photoreceptor layer. **Conclusion:** There is increase in apoptotic and autophagic markers expression in the RPE, choroid and photoreceptor layer in light damaged retina. In the ischaemic retina with 24 hours reperfusion there is sustained expression of Bax and caspase 3.

POS-WED-065

ACTIVATION OF VOLTAGE-GATED ION CHANNELS IN RABBIT RETINAL GANGLION CELLS FOLLOWING ELECTRICAL STIMULATION OF THE RETINA

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Several research groups have been developing prosthetic devices to restore vision to the profoundly blind. While it is known that electrical stimulation of the retina could elicit visual percepts, little is understood on the underlying neurophysiologic mechanisms evoked by the stimuli. We investigated how the voltage-gated channels in retinal ganglion cells (RGCs) shape the responses of these cells following electrical stimulation. Whole-mount retinas of NZ White rabbits were isolated, bathed with ~35 ºC Ames' Medium, and the RGCs targeted for whole-cell current or voltage clamp recordings. The electrical stimuli were applied via a multi-electrode array containing 40 x 40 µm platinum electrodes. To isolate the effects of the stimuli on RGC voltage-gated channels, we blocked the presynaptic inputs with a mixture containing CNQX, MK-801, L-AP4, picrotoxin and strychnine, to block AMPA/kainite, NMDA, mGluR6, GABA and glycin receptors, respectively. In more than half of the RGCs examined (n = 17/28) a cadmium-sensitive afterhyperpolarization was observed following electrical stimulation, indicating the activation of a calcium-activated potassium conductance. In many RGCs (n = 11/28) an afterdepolarization was evoked by the stimuli. This was suppressed by cadmium and Nifedipine. Thus the L-type calcium current was, at least in part, responsible for the depolarization. We also found present in most rabbit RGCs the hyperpolarization activated cationic current and the T-type calcium current. Activation of these currents by electrical stimulation resulted in long-duration subthreshold depolarization, and in some cases, also action potentials tens of milliseconds after the stimulus presentation. These results demonstrate that RGCs do not behave as simple spike generators following electrical stimulation, as often implicitly assumed in previous work. Instead, the stimuli activate several voltage-gated channels, which shape the responses of the stimulated RGCs over tens to hundreds of milliseconds, long after the short-duration electrical pulse has been delivered.

POS-WED-066

ABSENCE OF A FUNCTIONAL P2X7 RECEPTOR SLOWS PHOTORECEPTOR DEGENERATION IN THE RD1 MOUSE

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Purpose: Adenosine tri-phosphate (ATP) acts as a neurotransmitter by stimulating purinoceptors, which include the P2X ligand gated ion channels. Blockade of P2X receptors increases the survival of rod photoreceptors in the rd1 mouse model, through a mechanism likely to be mediated by the P2X7 subclass of receptors (P2X7-R). The aim of the current study was to investigate the role of the P2X7-R in mouse retinal degeneration. **Methods:** Quantitative RT-PCR was used to assess P2X7-R and one of the enzymes responsible for digestion of ATP, NTPdase1, mRNA expression in the retina of rd1 mice and C57Blk6 control mice (n=16 each) at postnatal day 14 (P14). The rd1 mouse line was crossed with a P2X7-R knock out mouse (P2X7-R-KO) to generate a P2X7-R-KO/rd1 mouse. Photoreceptor numbers were quantified and the inflammatory response was assessed by counting microglia in rd1 (n=6) and P2X7-R-KO/rd1 (n=6) mice at P14 and P18. **Results:** Both P2X7-R and NTPdase1 mRNA expression were more than doubled in Rd1 mice at P14 ($p<0.001$), implicating a role for this pathway in retinal degeneration. Rd1 mice lacking the P2X7-R had 30% more photoreceptors than the control rd1 mice at P14 ($p<0.01$). In addition, there were around 35% less microglia in the photoreceptor layer of P2X7-R-KO/rd1 mice at P14 and P18 ($p<0.01$) indicating a reduced inflammatory response in these mice. **Conclusion:** These results suggest blockade of the P2X7-R may be a useful therapy in slowing photoreceptor death in retinal degeneration. Inhibition of the P2X7-R may reduce photoreceptor death by blocking direct neurotoxicity mediated by extracellular ATP and indirect toxicity mediated by the inflammatory response of microglia.

POS-WED-067

LESIONS OF THE PRIMATE STRIATE CORTEX (V1) DURING INFANCY AND IN ADULTHOOD DIFFERENTIALLY ALTER THE INTERCONNECTIVITY OF THE MIDDLE TEMPORAL (MT) AREA AND VISUAL THALAMIC NUCLEI

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Purpose: To investigate the thalamic control of visual cortical maturation and how early perturbations alter normal development. **Methods:** Retinothalamocortical connectivity was visualized through intraocular injections of anterograde tracer and cortical injections of retrograde tracer into area MT a year after unilateral V1 ablation in postnatal day 14 (PD14, n=4) and adult (n=4) marmoset monkeys (*Callithrix jacchus*) and compared to neonatal (PD7-270, n=12) and adult control animals (n=2). Cerebral tissues were processed immunohistochemically to assist in the demarcation of thalamic nuclei and visual cortices, and to determine the colocalisation of fluorescently labelled area MT relay cells and retinal input in adjacent sections plotted using MDplot. Colocalisation was confirmed using the presynaptic marker synaptophysin and statistical analyses of resultant confocal images. **Results:** In the controls it was confirmed that relay cells to ipsilataral area MT were colocalised with labelled retinal ganglion cell terminals in PIm with there being an increase in labelling of binocular input to the PIm during development and prior to two months. Compared to the nonablated adult control and adult V1 ablated animals, neonatal removal of V1 resulted in the sparing of retinal input to PIm and an increase in the size of area MT. **Conclusion:** These data provide evidence of a putative pathway involving the pulvinar and area MT that may underpin the improved visual capacity observed in humans following a lesion of V1 early in life (prior to the closing of the critical period) compared with adults.

POS-WED-068

CHEMOKINE SIGNALLING MODULATES RETINAL FUNCTION IN A LIGHT DAMAGE MODEL OF DEGENERATION

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Purpose: The chemokines MCP-1 (Ccl2) and fractalkine (CX3CL1) have been shown to participate in a diverse range of inflammatory processes, particularly as they influence the course of microglia, which are the subject of much interest due to their multifaceted role in the CNS. We aimed to determine the role of these chemokines in modulating retinal function in light damage (LD) induced retinal stress. **Methods:** Mice lacking MCP-1 (n=6) (C57bl6 background) and the receptor for fractalkine, CX3CR1 (C57bl6 and balb-c background) were subjected to acute LD of 10,000lux for 12 hours (C57bl6) and 6 hours (balb-c). A twin-flash electroretinogram (ERG) was taken before LD and afterwards (at 24 hours and 10 days) to assess the function of the rod and cone pathways. **Results:** There was no significant difference in retinal function between the Ccl-2 and CX3CR1 knock-outs on the pigmented C57bl6 background before or after LD. In the CX3CR1 knock-out mice (n=18) on the albino background before LD, there was a significant reduction in the rod ERG response when compared with the balb-c controls (n=30). Following LD both the balb-c controls and CX3CR1 knock-outs showed a significant reduction in rod pathway function, however the CX3CR1 knock-outs were less severely affected, showing greater rod pathway function post LD at both 24hrs and 10 days post-LD. **Conclusion::** This suggests that fractalkine activation of CX3CR1 might contribute to photoreceptor damage during LD. Blockade of CX3CR1 may provide a novel mechanism for reducing functional loss in retinal degeneration.

POS-WED-069

THALAMIC ANATOMY AND BIOCHEMISTRY IN NEUROPATHIC AND NON-NEUROPATHIC CHRONIC PAIN SYNDROMES

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Purpose: Chronic pain exists in different forms and aetiologies, many of which are not well understood. Evidence suggests that changes within higher brain centres, in conjunction with peripheral changes, are also important for the maintenance and/or development of some chronic pain conditions. In particular the thalamus has been shown to display anatomical, biochemical and physiological changes, predominantly in neuropathic pain. The aim of this investigation was to use voxel based morphometry (VBM) and magnetic resonance spectroscopy (MRS) to determine whether changes in regional neuroanatomy and biochemistry occur in two orofacial pain conditions: Trigeminal Neuropathic Pain (TNP) and Temporomandibular Disorders (TMD). **Methods:** Twenty one TNP, 20 TMD and 47 healthy controls underwent MRI acquisition which included a 3D, T1-weighted anatomical image set. In addition, a subset (11 TNP, 11 TMD and 10 controls) underwent single-voxel spectroscopy. SPM5 and jMRUI were used for the VBM and MRS analysis. **Results:** VBM analysis revealed that TNP subjects had significant ($p<0.05$) regional grey matter volume changes in a number of brain regions, notably a significant decrease in thalamic volume contralateral to their pain side. Furthermore, MRS analysis revealed that this decrease in thalamic volume was associated with a significant decrease in neural viability (decreased NAA:Creatine). In contrast, TMD patients did not display any significant change in thalamic grey matter volume or neural viability. **Conclusion:** Our data shows that neuropathic, but not non-neuropathic pain, is associated with significant changes in brain structure and biochemistry. In particular, neuropathic pain is associated with significant reductions in the volume and neuronal viability of the thalamus.

POS-WED-070

COMBINING CELL-BASED THERAPIES AND NEUROPROSTHESES TO PROMOTE NERVE SURVIVAL

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Purpose: Spiral ganglion neurons (SGNs) in the deafened cochlea undergo continual degeneration ultimately resulting in cell death. The exogenous application of neurotrophins (NTs) can prevent SGN degeneration, with the survival effects enhanced by chronic intracochlear electrical stimulation (ES) from a cochlear implant. However, techniques available to administer NTs have limited clinical applicability, thus restricting the use of NTs in treating neurodegenerative diseases. Methods: We have used a cell-based technique to provide NTs in a clinically viable manner that can be combined with cochlear implant use. Neonatal cats were ototoxically deafened systemically and at two months of age were unilaterally implanted with encapsulated NT-producing cells and a cochlear implant (Cochlear CI24) to deliver environmentally-derived ES. Animals received chronic ES only (n=5), NTs without chronic ES (n=6) or NTs in combination with chronic ES (n=6) over 6-8 months. In all cases the opposite ear served as a deafened control. A normal hearing control cohort (n=7) were not implanted. Results: Chronic ES alone did not result in greater SGN survival when compared to the contralateral cochlea. NT treatment alone resulted in significant SGN survival in the upper basal cochlear region. Importantly, chronic ES in combination with NT provided significant SGN survival throughout the basal and lower middle regions. Electrical thresholds were stable over the treatment period for all groups. Conclusion: We conclude that cell-based NT delivery is clinically viable and effective in preventing SGN degeneration over extended durations of deafness. These findings have important implications for therapies that deliver therapeutic drugs safely to the cochlea.

POS-WED-071

ARE RETINAL GANGLION CELL INTRINSIC PHYSIOLOGICAL PROPERTIES CONSERVED?

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Different aspects of the visual scene (e.g. form, colour, motion) are encoded in the retina in many parallel pathways. These features are extracted through the complex synaptic array of the more than 60 different retinal cell types. Ultimately, this information must be encoded into the spike trains of the 15-20 different retinal ganglion cell types whose axons reach a similar number of central brain nuclei. Previously, we have shown that the intrinsic physiological properties of RGCs vary widely. For example, cat alpha RGCs are capable of spiking in excess of 300Hz whereas the kappa cell can only sustain 40Hz (O'Brien et al., 2002). We have now completed a comprehensive study of rat RGCs (n = 85) which has a diversity in their intrinsic physiological properties similar to that found in cats. We wondered, therefore, whether these intrinsic physiological properties are conserved among the two species. Using a hierarchical cluster analysis (SPSS) our data suggest that some well characterized cat RGC types have close relatives in the rat retina: (e.g. cat/rat: alpha/A2, zeta/B4). In contrast, some cat RGCs seemed to be unrelated to all rat RGCs (e.g. beta, theta). Using a strict criterion, our cluster analysis suggests that when using 8 intrinsic properties (Resting potential, Input Resistance, Time Constant, Maximum Frequency, Spike width, Steady-State Frequency, Frequency Adaptation, Anomalous Rectification) exclusively, 5 different cell classes including many different morphological types can be resolved. As the spiking output of RGCs ultimately limits what information they can carry, our data suggest that five different 'channels' may have evolved to support different forms of information transfer from the eye to the brain.

POS-WED-072

BIONIC VISION - A RETINAL NETWORK MODEL

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Purpose: In this study we describe a 3D computational model of the retinal network incorporating active neural layers and passive retinal properties, in order to simulate the response of the retina to electrical stimuli from a vision prosthesis. Simulations were performed to investigate retinal ganglion cell (RGC) spiking activity in response to external electrical stimuli from epiretinal and suprachoroidal electrodes. **Methods:** A 3D finite element model of the retina was developed incorporating all major neural layers from the vitreous to the choroid, with these layers treated as passive volume conductors. The active RGC layer was modelled based on the ionic formulation of Fohlmeister & Miller, 1997. The intracellular potential was resistively tied to a resting potential representing distal portions of the neuron. This allows for local excitation of RGCs without the spread of excitation to neighbours, essential in eliciting focal percepts. Bipolar and amacrine cells were modelled passively and provided input to the RGCs via a synaptic current determined by integrating over the area of the dendritic receptive field. **Results:** Simulations were conducted using both monophasic and biphasic cathodic first stimulus current waveforms. Current threshold densities of 52μC/cm^2 and 76μC/cm^2 for epiretinal and suprachoroidal electrode placements respectively were obtained, these are consistent with published data by Sekirnjak, 2008 and Wong, 2009. **Conclusion:** The novel retinal model presented in this study was able to simulate patterns of spiking activity in RGCs arising from electrical stimulation from suprachoroidal and epiretinal electrodes of a vision prosthesis. Thresholds were comparable to previous published findings.

POS-WED-073

THE ROLE OF NEUROTROPHINS IN OBSTRUCTION-INDUCED BLADDER OVERACTIVITY

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Purpose: To determine whether bladder overactivity (in early stages of obstruction) is due to enhanced excitability of bladder afferents, by the sensitizing action of NGF and/or BDNF. **Methods**: Partial bladder outflow obstruction was produced by narrowing the urethra for duration of 7-12 days. Single unit stretch-sensitive bladder afferents were recorded by conventional extracellular recording technique *in vitro*. The effect of antiserum against BDNF or NGF was tested by i.p. injections of 10 µl/g at first and 5 µl/g at fourth and seventh days after obstruction. **Results**: The frequency of conscious voiding was increased after bladder obstruction (2.8 ± 0.2 per 2 hrs, n=5), compared with control (1.6 ± 0.3 per 2 hrs, n=10, P<0.05), but not in obstructed mice treated with antiserum against BDNF or NGF (n=5). In anesthetized mice, the frequency of distension-induced reflex bladder contractions was significantly increased after obstruction (1.14 ± 0.14 per min, n=4), compared with control (0.64 ± 0.1 per min, n=5, P<0.05), but not in obstructed mice treated with antibodies against NGF or BDNF (n=5). The excitability index of bladder afferents was significantly higher in obstructed preparations: at 20 Hz, 77 ± 11% (n=5) of spikes followed stimulation in obstructed preparations, while in control 47 ± 6% (n=4, P<0.05). In obstructed mice, intravesical pressure responses to electrical field stimulation were significantly reduced (n=5). The cholinergic component was reduced from 45 ± 18% (n=4, control) to 26 ± 2.8% (n=5, obstructed, P<0.001), while the purinergic component was unaffected. **Conclusion**: The results suggest that increased production of NGF and BDNF during bladder obstruction could sensitize bladder afferents that may lead to the development of bladder overactivity.

POS-WED-074

CONTRALATERAL PRIMARY VISUAL CORTEX AND CLASSICAL AND EXTRA-CLASSICAL RECEPTIVE FIELD INTERACTIONS IN CAT'S AREA V2

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In domestic cats, neurones located in the transition zone between visual areas 17 (V1) and 18 (V2) receive substantial direct input from topographically corresponding regions in the opposite hemisphere. This commissural (callosal) input to primary visual cortex appears to be excitatory and contributes to binocularity of cortical neurons[1]. **Purpose:** To examine if interhemispheric input to primary visual cortex contributes to modulatory interactions between spike-generating classical receptive fields (CRFs) and the silent suppressive extraclassical receptive fields (ECRFs). **Methods:** We recorded single neuron activity from area V2 of anaesthetized cats. Achromatic sine-wave modulated drifting gratings were used to determine the extent of the CRFs. Contralateral V1/V2 border region was reversibly inactivated by cooling it to 10°C and interactions between CRFs and ECRFs were tested by varying the orientation of the gratings presented to ECRFs. **Results:** In most cells (15/24; 8 simple, 7 complex) with CRFs located within 6° of the vertical meridian, inactivation of the contralateral V1/V2 border region resulted in significant reductions or increases in the magnitude of spike-responses to high-contrast CRF-confined stimuli. In most cases (8/10; 3 simple, 5 complex) the reductions were accompanied by significant changes in the relative strength of ECRF modulation of CRF-induced spike-responses. The increases in the magnitude of responses to CRF-confined stimuli however were not accompanied by significant changes in the relative strength of ECRF-induced modulation. **Conclusion:** Callosal inputs from primary visual cortices make significant contributions not only to the magnitude of CRF-induced spike-responses but also to the ECRF-induced modulation of spike-responses of V2 neurones. [1]Blakemore C et al., (1983) J Physiol (Lond) 337, 331- 349.

POS-WED-075

CHANGES IN SKILLED FORELIMB MOVEMENT AFTER LATERAL FUNICULUS LESIONS IN THE RAT: ANATOMICAL AND BEHAVIOURAL DISSOCIATIONS

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Lesion studies in the rat showed that the integrity of the lateral funiculus (LF) is critical for the execution of skilled forelimb movements (e.g., Schrimsher and Reier, 1993; Muir et al., 2007; Stackhouse et al., 2008; Kanagal and Muir, 2009). In these studies, however, detailed histological analysis of the extent of the individual lesions is not always provided. Moreover, the size of the LF lesions varies from one study to the other thus making these data difficult to interpret from an anatomical point of view. **Purpose:** The aim of this study was to systematically compare the effect of different sizes of LF lesions on skilled reaching with particular attention to the movements of the paw and digits. **Methods:** Rats (n = 17) that were pre-trained on the skilled reaching task received LF lesions and their pre- and post-operative performance on this task was compared. Based on strict histological criteria, they were then assigned to a large, medium or to a small LF lesion group. **Results:** Large LF lesions significantly impaired the ability to perform arpeggio movements where digit 5 first contacts the shelf followed by digits 4 to 2. Such lesions also significantly impaired the grasp movement where the digits flex and close around the food pellet. Medium LF lesions significantly impaired the placement of digits 4 to 2 on the shelf and with the ability to flex the fingers around the food. Small lesions only significantly disrupt the ability to place digits 4 to 2 successively on the shelf. **Conclusion:** The results will be discussed in terms of the different fibre pathways running within the LF that are disrupted by these different lesions.

POS-WED-076

PROJECTIONS TO THE MESENCEPHALIC RETICULAR FORMATION IN MICE

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In mice, the mesencephalic reticular formation (mRt) has been shown to be involved in many behaviors, including eye movement, eating, urination, and defecation. However, there is scant information on the connectivity of the mRt in rodents. **Purpose:** This study explores the projections to the mRt. **Methods:** In mice (n=10) and rats (n=6), the retrograde tracer FluoroGold was injected into the mRt and the retrogradely labelled neurons were mapped. **Results:** Forebrain afferents to mRt in mice arise chiefly from the motor cortex, the insular cortex, the secondary visual cortex, the ventral pallidum, the mediodorsal thalamic nucleus, the zona incerta, and the periventricular hypothalamic nucleus. Brain stem areas that strongly project to the mRt are the periaqueductal grey, the intermediate and deep gray layers of the superior colliculus, the inferior colliculus, the substantia nigra, the posterodorsal raphe nucleus, the red nucleus, the pontine reticular nucleus, the parabrachial pigmented nucleus, the cochlear nuclei, the spinal vestibular nucleus, the gigantocellular nucleus, the facial nucleus, and the dorsal nucleus of the inferior olive. Smaller numbers of labelled neurons are found in the somatosensory cortex, the septal area, the paraventricular hypothalamic nucleus, and the spinal. The pattern of the retrogradely labelled neurons in rats is largely similar to that seen in mice. **Conclusion:** Most of the structures that project to the mRt are known to play a role in motor control, which is consistent with physiological studies showing the involvment of mRt in many motor functions. The projections from the paraventricular hypothalamic nucleus indicate that the mRt is also involved in neuroendocrine functions.

POS-WED-077

CHONDROITINASE TREATMENT REVEALS POTENTIAL STRIATAL CRITICAL PERIOD

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Purpose: Sensory cortex is characterized by a developmental 'critical period', during which neural circuits are particularly sensitive to changes in activity. Little is known whether similar epochs exist in motor pathways. Recent work from our lab has shown that a marker for the closure of sensory critical periods, perineuronal nets (PNNs) composed of chondroitin sulfate proteoglycans (CSPGs), begin forming in the mouse striatum during the first and second postnatal weeks, around the time pups begin to exhibit coordinated movements. In order to test whether PNNs demarcate a similar consolidation of striatal networks vital for controlling movements, we enzymatically removed these striatal CSPG structures in one week old pups, and determined whether this treatment resulted in measurable changes in motor behaviour. **Methods:** Motor behaviour of Chondroitinase ABC (ChABC) treated (n = 9) and untreated (n = 13) pups were assessed using an open field test and free swimming task. **Results:** Treated subjects exhibited significantly longer latencies to reach pre-determined 'boundaries' within the open field (Wilcoxon rank-sum test, p = 0.04). ChABC treated animals tended to also exhibit less coordinated swimming behaviour. **Conclusion**: The removal of striatal PNNs during early postnatal development delays the maturation of motor behaviours. These observations provide evidence that (1) the emergence of these simple controlled movements may be dependent on the formation of these CSPG structures in the striatum specifically, and (2) striatal PNNs serve as a marker for network consolidation or the closing of a 'critical period' in this vital motor nucleus.

POS-WED-078

KINEMATIC AND CORTICOMOTOR CHANGES ASSOCIATED WITH REPEATED MAXIMAL FINGER MOVEMENT TASK IN HEALTHY INDIVIDUALS

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Exploration of limitations to the human motor system have largely been confined to force measurements and sequential-related movements. Recently, we reported that a finger flexion-extension task performed at maximal voluntary rate (MVR) could not be sustained for 10 seconds, and that this was not due to muscle fatigue, indicating a breakdown in central motor control. In the present study, we hypothesized that if the loss of motor control was central then it should be possible to improve from a period of practice. The aims of the present study were to determine if short-term practice can improve movement kinematics of a 10-second index finger flexion-extension MVR task, and to investigate any related changes in corticomotor excitability to the first dorsal interosseous muscle. Nine subjects performed 6 sets of the MVR task with a 5-minute rest period between each set. Transcranial magnetic stimulation (TMS) was applied 10 seconds and 2 minutes after each set. Mean starting rate (first 2 secs) increased 5% after 6 sets of practice ($p<0.05$). For each set, although overall rate and amplitude declined during the 10 seconds of the task, their decline was less by the 4th set. MEP amplitude increased significantly after each set (16% and 13%; 1st and 2nd TMS recording respectively; $p<0.05$). The results indicate that performance of a MVR task can improve over a relatively short period of practice, and this is associated with a steadily increasing level of corticomotor excitability. The increase in excitability may reflect neuroplastic changes associated with motor learning.

POS-WED-079

SPATIAL CHARACTERISATION OF THE MOTOR NEURON COLUMNS INNERVATING THE MUSCLES OF THE RAT FORELIMB

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We are currently working on a project that aims at the viral-mediated delivery of therapeutic genes to populations of motor neurons in a rat model of spinal cord injury. We seek to take advantage of the natural retrograde transport machinery of neurons to deliver these genes via intramuscular injections. To achieve gene delivery into specific segments of the spinal cord, knowledge about the precise relationship between the different muscles of the forelimb and the location of the motor neurons that innervate them must first be established. **Purpose:** The aim of the present study was to create a topographic map of the motor neurons that innervate the main muscles of the forelimb. **Method:** Ten muscles from the shoulder, the upper and lower parts of the forelimb were injected with Fluoro-emerald or Fluoro-Gold. For each rat (n=27) the entire cervical spinal cords was divided into 2-segment blocks and the tissue was cut longitudinally. The tissue sections were subsequently scrutinised to see the presence of labelled motor neurons using epifluorescence microscopy. For each muscle, the retrogradely labelled motor neurons were plotted and a 2-D reconstruction was obtained by stacking the plots. **Results:** This analysis revealed that the motor neurons innervating the muscles of the forelimb are organised into columns that span across several segments of the spinal cord. Although discrete these individual columns exhibit a substantial degree of overlap on the rostro-caudal axis. The rostro-caudal topography of these motor neuron columns reflects the proximo-distal organisation of the muscles of the forelimb. **Conclusion:** This map constitutes a valuable guide for the selection of the appropriate muscle(s) for the delivery of therapeutic genes into specific segments of the cervical spinal cord.

POS-WED-080

LOCALIZATION OF THE SENSORY NEURONS UNDERLYING VISCERAL PAIN FROM THE TERMINAL GASTROINTESTINAL TRACT

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Purpose: The location of the sensory neurons in dorsal root ganglia that underlie the activation of visceral pain pathways from the terminal gastrointestinal tract are unclear. Methods: DiI retrograde neuronal tracer was injected into three different sites along the colon and rectum of anaesthetized mice and the location of retrogradely labeled DRG neurons identified 7 days after dye injection. DRGs were harvested from the entire cervical to sacral region of spinal cord and serial cryosections were made (12microns in depth) from fixed DRGs. The Distribution and absolute number of DRG neurons was then evaluated. Results: Single DiI injections (300microL) into the terminal rectum, at a distance of 1-3mm from anus (in unstretched colonic preparations) exclusively labelled DRGs in the lumbar and sacral spinal cord (n=4). DRGs in S2 were the primary site of labeling. In lethal spotted (ls/ls) mutant mice, which develop colorectal aganglionosis, there was a 60% reduction in the number of DRGs labeled (n=5), but the same distribution of DRGs existed. When DiI injections were made 9-19mm from the anus (again in unstretched preparations), surprisingly, the same distribution of DRGs was obtained (n=4). That is, no DRGs were labeled in thoracic spinal cord. Only when dye injections were made in the mid-proximal colon (a distance of 30-75mm from anus) were DRGs labeled in the thoracolumbar region of spinal cord (n=3); where no sacral DRGs were detected. Conclusion: These results show that stimulation of terminal 20mm of mouse colorectum, activates sensory neurons exclusively in the lumbar sacral region of the spinal cord. p.

POS-WED-081

CONNEXINS IN THE HUMAN COLON: ARE THEY TO BLAME FOR THE DYSMOTILITY OF THE COLON IN PATIENTS WITH SLOW TRANSIT CONSTIPATION?

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Slow transit constipation (STC) is a colonic dysmotility, characterised by the inability to defecate more than once every 2-3 weeks. STC predominantly occurs in females of childbearing age. To date, no efficacious treatment has been successfully developed due to the lack of knowledge on the mechanism of this disorder. Connexins are “gap junction proteins” which bridge the extracellular space thus creating connections between cells. **Purpose:** Here, we examined whether abnormal expression of connexin proteins occurs in the colon of STC patients, which may lead to a loss of coordination of colonic motility. **Methods:** Sigmoid colon segments were obtained from age-matched female patients (aged 23-69 years) undergoing resection for STC (n=11), or for carcinoma (normal colon, 30-68 years, n=12). Real-time RT-PCR, immunohistochemistry and western blotting were used to detect the mRNA and protein expression of connexin 26, 43 and 45. **Results:** In STC mucosa, there was a 2.7-fold and 5-fold down-regulation of Cx43 and Cx45, respectively (both $P < 0.01$, Mann Whitney test) compared to control. Cx26 expression was unaltered in STC mucosa. In contrast, STC muscle showed no change in Cx43 and Cx45 mRNA expression, whereas Cx26 mRNA was not detectable in either control or STC muscle. These results were consistent with the findings from western blotting, showing a 6-fold ($P<0.001$) and 3-fold ($P<0.01$) down-regulation of Cx43 and Cx45 proteins, respectively, in STC mucosa, but no change in STC muscle. Immunoreactivity for Cx43 and Cx45 was widely present in most cell types; however in the enteric ganglia dense Cx45 staining was observed, but Cx43 expression was negligible. In STC, reduced Cx43 and Cx45 immunoreactivity appeared to occur only in the mucosal crypts. **Conclusion:** Altered connexins in the mucosa may result in faulty cell communication which affects sensory impulses of mucosal origin, thus impairing the colonic peristaltic reflex and/or the colonic migrating motor complex in STC patients.

POS-WED-082

OREXIN MICROINJECTIONS IN THE MEDULLARY RAPHE INCREASE HEART RATE AND ARTERIAL PRESSURE BUT DOES NOT REDUCE TAIL BLOOD FLOW

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Orexinergic projections to the lower brainstem are likely to be involved in the production of autonomic responses associated with arousal. The rostral ventromedial medulla (RVMM) is a premotor sympathetic centre that controls heart rate, skin (tail) vasomotor tone and to a lesser extent, arterial pressure. In the anaesthetised rat, orexin has been shown to increase heart rate, but not arterial pressure when injected in this area. Skin vasomotor response has not been investigated yet. **Purpose:** To observe the effect of orexin injection into the RVMM on heart rate (HR), mean arterial pressure (MAP) and tail skin blood flow. **Method:** Rats were implanted with radio-telemetric probes and a cannula aimed at nucleus raphe pallidus. They were injected with 0.4ul of either saline or orexin-A (3 and 30 pmole) and returned to their home box. Changes in tail blood flow were obtained from infrared thermographic recording of changes in temperature in the skin of the tail (TTail). **Results:** Orexin-A (30 pmole) into the RVMM (n=7) had no effect on locomotor activity ($p=0.068$) but increased HR and MAP ($p<0.05$). Interestingly, there was no effect on TTail indicating that tail blood flow was not reduced. **Conclusion:** The results suggest that orexinergic projections to the RVMM can drive the local premotor sympathetic neurons that control HR and MAP but not those that control skin vasomotor tone. Thus, the RVMM premotor sympathetic neurons that drive skin vasomotor tone do not appear to be controlled by orexin during arousal.

POS-WED-083

TRANSNEURONAL TRACING OF AIRWAYS RELATED SENSORY CIRCUITRY USING HERPES SIMPLEX VIRUS 1, STRAIN H129

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Herpes simplex virus-1 (HSV-1) undergoes both retrograde and anterograde transneuronal movement along synaptically connected neurons. The H129 strain of HSV-1 is unique as it travels primarily in the anterograde direction which makes it ideal for mapping sensory pathways in the CNS. **Purpose:** To assess the utility of the H129 strain for mapping the central organisation of the sensory circuitry arising from the extrathoracic trachea. **Methods:** Under Isoflurane anaesthesia rats (n=22) were inoculated with 10µl of the H129 strain into the extrathoracic tracheal lumen at titers ranging from $7x10^4$ to $7x10^7$ pfu/ml and allowed to recover for up to 120 hours. All relevant tissues along the neuroaxis were processed for HSV-1 immunoreactivity. **Results:** The optimal titer for transneuronal infection was $7x10^7$ pfu/ml. After 24 hours H129 infection appeared in the vagal ganglia and the number of infected cells progressively increased thereafter. After 96 hours robust H129 infection was evident in vagal and trigeminal sensory nuclei in the brainstem and could be detected as far rostrally as the thalamus. After 120 hours infection was detected in cortical regions including the cingluate cortex. The was no evidence of transneuronal labelling of autonomic motor pathways. **Conclusion:** These data show that the H129 strain is a useful tool for mapping visceral sensory circuitry and will provide valuable insight into the higher order brain regions involved in sensing airways irritation.

POS-WED-084

KISSPEPTIN NEURONES OF THE RAT PERIVENTRICULAR NUCLEUS ARE SENSITIVE TO INSULIN STIMULATION

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The relationship between nutrition and fertility has long been recognized. Insulin signalling is one nutritional factor that has been shown to alter reproductive capacity. Moreover, pathological states such as diabetes mellitus and polycystic ovary syndrome commonly present with concurrent insulin dysregulation and reproductive anomalies. However, the locus of insulin sensitivity for regulation of fertility remains obscure. As direct regulators of the reproductive axis, kisspeptin-expressing neurones, found in the periventricular (PeVN) and arcuate (ARC) nuclei of the rat hypothalamus are a potential locus of insulin sensitivity for nutritional control of fertility. **Purpose**: the current study aimed to test if kisspeptin neurones are able to respond to insulin in the rodent hypothalamus. **Methods**: female Sprague-Dawley rats were administered a 2 µl bolus containing 4 mU of insulin (n=5) or saline vehicle (n=5) via a previously implanted intracerebroventricular cannula. Anaesthetised animals were trans-cardially perfused with 4% paraformaldehyde 30 minutes later. Brains were removed and processed for immunohistochemistry. Sections containing hypothalamic regions underwent dual-label peroxidase immunohistochemistry for an active component of the insulin-signalling pathway (phosphorylated-AKT; pAKT) and kisspeptin. **Results**: in the PeVN of insulin-stimulated rats, 69.8 ± 8.1% of kisspeptin-positive neurones also expressed pAKT; a significant increase above basal colocalisation in the same region (17.6 ± 6.5%; Student’s t-test; $p < 0.001$). Conversely, colocalisation was low in the ARC of both saline (3.7 ± 1.7%) and insulin-treated (8.9 ± 2.5%) animals (Student’s t-test; $p > 0.05$). **Conclusion**: these results indicate that a population of kisspeptinergic neurones in the rat hypothalamus are sensitive to insulin and that this may provide a mechanism for insulin’s regulation of the reproductive axis.

POS-WED-085

ULTRADIAN RHYTHM IN OREXIN DEFICIENT MICE

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Purpose: Brown adipose tissue, body and brain temperature suddenly increases in a episodic manner, approximately every 95 min during the active phase of the circadian cycle. This ultradian rhythm associated with tachycardia, pressor response, locomotor activity and arousal brain signal[1]. Orexin-producing neurons in the lateral hypothalamus are important for arousal stability. We investigated whether eliminating orexin affects ultradian rhythms in body temperature, heart rate and locomotor activity by using orexin deficient mice. **Methods**: We used orexin knockout (orexin-KO) mice[2], orexin/ataxin-3 mice[3], in which postnatal death of orexin-producing neurons occurs, and control wild mice. A telemetry probe for measurements locomotor activity, body temperature and ECG (Data Science International, ETA-F10) was implanted under isoflurane anesthesia. At least one week later, conscious unrestrained mice were placed in a quite constant temperature (26ºC), and then all variables were measured for 36 hours under 12/12hrs light-dark cycle. Cosinor analysis was used to assess ultradian periodicity and its amplitude. **Results**: In wild control mice, during the dark active period, ultradian episodic increases in locomotor activity was observed, and correlated with similar changes in body temperature and heart rate. The ultradian periodicity in locomotor activity was 117±18 min (mean±SEM, n=3), and its amplitude was 84±13 (arbitrary unit). In orexin deficient mice, the amplitude of the ultradian rhythms in locomotor activity was significantly reduced to 45% ($P<0.05$, n=3) in orexin-KO mice and to 41% ($P<0.05$, n=3) in orexin/ataxin-3 mice, comparing with wild-mice ultradian amplitude. **Conclusion**: These results suggest that brain orexin-system contributes to the ultradian rhythm. (1) Ootsuka et al., Neuroscience 164:849, 2009. (2) Chemelli et al., Cell 98:437, 1999. (3) Hara et al., Neuron 30:345, 2001.

POS-WED-086

SUBPOPULATIONS OF SYMPATHETIC PREGANGLIONIC NEURONS AND ADRENAL CHROMAFFIN CELLS ARE ACTIVATED AFTER GLUCOPRIVATION

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Administration of glucose analog 2-deoxy-D-glucose (2DG) causes glucoprivation resulting in the selective release of adrenaline. Adrenal chromaffin cells, the source of adrenaline, are innervated by sympathetic preganglionic neurons (SPN) of the spinal cord with the greatest innervation occurring from levels T4-T10 and lesser innervation arising from C8-T3 and T11-L2 levels. About 80% of chromaffin cells have synthesizing enzymes for adrenaline, indicating that a large proportion but not all cells may be active after glucoprivation. **Purpose:** Our aim is to identify the functional phenotypes of SPN which are involved in adrenaline release by determining the chemical code expressed by these cells following the administration of 2DG. Firstly, we will determine the SPN population involved and then identify their chemical phenotype. **Methods:** Glucoprivation was induced by injection of 2DG (400mg/kg) in adult male Sprague Dawley rats (n=4) and compared to rats that received saline (n=4). SPN were identified using ChAt or vAchT immunohistochemistry. In combination with immunohistochemistry for Fos, CART protein and in situ hybridisation for enkephalin and PACAP mRNA will be performed to identify chemical phenotypes of SPN activated by 2DG that project to the adrenal medulla. **Results:** In T4-T10 sections, 22.5% (1203 of 5343 counted cells) SPN expressed Fos immunoreactivity in animals receiving 2DG whereas only 0.004% (20 of 4989 counted cells) occurred in saline treated animals. Intense Fos activation is seen in most but not all adrenal chromaffin cells of 2DG animals (n=3). **Conclusion:** So far results indicate specific populations of SPN and adrenal chromaffin cells are activated after glucoprivation. Further neurochemical coding is being conducted using retrograde tracing to identify adrenally projecting SPN activated at spinal levels C8-L2 to determine the involvement of several functional phenotype populations in adrenaline release.

POS-WED-087

INTRATHECAL NEUROMEDIN U CAUSES SYMPATHETICALLY MEDIATED BIPHASIC CHANGES IN BLOOD PRESSURE AND INCREASES RESPIRATORY DRIVE

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Neuromedin U (NMU) is a brain–gut peptide, which plays regulatory roles in feeding, energy homeostasis, stress, smooth muscle contraction, blood flow, pain and adrenocortical function via two receptors, NMUR1 and NMUR2. While several peripheral activities have been described for NMU, its role in the CNS remains poorly understood. This study was undertaken to determine if NMU modulates sympathetic vasomotor output at the spinal level. Experiments were conducted on urethane anaesthetised, vagotomised and artificially ventilated Sprague-Dawley rats (n = 21). Intrathecal injections of NMU caused a biphasic response, initially a brief dose-dependent hypertension and sympathoexcitation followed by a prolonged hypotension and sympathoinhibition. The maximum excitatory and inhibitory effects were observed at 20 nmol with increases in mean arterial pressure (MAP) and splanchnic sympathetic nerve activity (sSNA) of 27 ± 4 mmHg and 29 ± 5 % from baseline and decrease in MAP and sSNA of 33 ± 4 mmHg and 41 ± 5 % from baseline respectively. NMU also dose-dependently increased respiratory drive as indicated by a rise in phrenic nerve amplitude (PNamp) of 30 ± 4 %, and increase in neural minute ventilation. These findings demonstrate NMU causes sympathetically mediated biphasic effects in MAP and increases respiratory drive.

POS-WED-088

CHANGES IN RFAMIDE-RELATED PEPTIDE-3 (RFRP-3) GENE EXPRESSION DURING LACTATION

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A hypothalamic RFamide neuropeptide that directly inhibits GnRH neuronal activity was recently discovered in birds and mammals. In our previous study, we showed that the mammalian ortholog, RFRP-3, is located in neurons scattered within the dorsomedial hypothalamus (DMH) of rats which project throughout the brain, but not to the neurosecretory zone of the median eminence (Rizwan et al 2009, Endocrinology 150: 1413-20). A study analysing lactation effects on RFRP expression has not been done, although RFRP expression has been shown to be increased in another state of infertility, the anoestrous sheep (Smith et al, 2008). **Purpose:** We hypothesized that RFRP gene expression would be up-regulated in lactating animals in response to high levels of circulating prolactin. **Methods:** Lactating (day 10) rats were given either bromocriptine (0.6 mg/kg sc) to suppress prolactin secretion or vehicle (sesame oil) every 12 hours (5 doses) and compared with age-matched diestrous rats (n=8-9). We also included lactating rats (day 10) with the pups removed for 6 h to examine the role of suckling (circulating prolactin levels were maintained at normal levels with ovine prolactin (4 mg/kg sc). The DMH was micropunched for RFRP mRNA analysis by quantitative RT-PCR. Serum rat prolactin concentration was measured to confirm bromocriptine effect. **Results:** In contrast to our hypothesis, RFRP gene expression was significantly down-regulated in the DMH of vehicle-treated and non-suckled lactating rats compared to diestrous rats ($p<0.05$). No difference in RFRP mRNA levels was observed in rats where prolactin secretion was suppressed with bromocriptine compared to diestrous rats. **Conclusion:** These findings suggest a role of prolactin in suppressing RFRP levels during lactation.

POS-WED-089

INVESTIGATION OF MECHANISMS UNDERLYING THE COLONIC MIGRATING MOTOR COMPLEX IN MICE

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Background: Resection of the colon is a common surgical treatment for a number of diseases including cancer and Hirschsprung's disease. Little is known, however, of the post-operative consequences of this treatment for colonic motility. The spontaneous behaviour of shortened segments of colon was investigated using preparations of mouse colon in vitro. ***Methods***: Spatiotemporal maps were generated from video images of spontaneous motility of adult mouse colon using a video camera positioned above an organ bath. The effects of the nitric oxide synthase inhibitor, NOLA (100 μM), and the voltage-gated sodium channel blocker, tetrodotoxin (TTX; 1 μM), were examined to reveal the influence of surgical resection on colonic motility. ***Results***: Colonic migrating motor complexes (CMMCs), which are cyclical, spontaneous, anally-propagating contractions generated and regulated by the enteric nervous system, were observed in segments of whole mouse colon, and in separated proximal and distal halves of the colon. Two preparation techniques were used – pinned preparations and cannulated preparations to control for differences in tissue preparation. NOLA (100 μM) significantly reduced the interval between successive CMMCs in whole length colon and cannulated proximal segments, but not in pinned proximal, pinned distal or cannulated distal segments. All propagating contractions were abolished by TTX. ***Conclusions***: CMMCs can be generated in isolated segments of proximal and distal colon. These respond differently to NOLA depending on the preparation technique used. These data suggest that some interruption in the neural circuits mediating propagating motility patterns occurs when half of the colon is surgically removed.

POS-WED-090

ARE 5-HT3 AND 5-HT4 RECEPTORS REQUIRED FOR THE GENERATION OF PERISTALSIS AND PROPAGATION OF COLONIC CONTENTS?

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Purpose: It has been proposed that endogenous release of serotonin (5-HT) from the intestinal mucosa; and subsequent activation of 5-HT3 and 5-HT4 receptors are essential for peristalsis and the propulsion of colonic contents along the large intestine of laboratory animals. This concept is important because it has been used as a foundation for the development of leading therapies for human patients with irritable bowel syndrome (IBS). The aim of this study was to determine whether antagonists of 5-HT3 and/or 5-HT4 receptors have a similar inhibitory effect on peristalsis, (as is seen in control preparations), when the mucosa and submucosal plexus have been removed from the colon. Methods: Video imaging of fecal pellet propulsion was made from isolated segments of guinea-pig distal colon and the propagation velocities determined from spatiotemporal maps. Results: Peristalsis was reliably evoked in segments of colon with mucosa and submucosal plexus removed (mean control velocity: 1.73mm/s; n=7). In mucosa and submucosal plexus-free preparations, the combined application of Granisteron (1μM) and SDZ 205-557 (1μM) did not prevent the initiation or propagation of peristalsis (nor fecal pellets), as previously suggested. In control (undissected) preparations (i.e. mucosa intact), there was similarly no blockade of peristalsis or abolition of pellet propulsion (control velocity: 2.5mm/s; n=9) compared with 3.08mm/s (n=6) in the combined presence of both antagonists. Granistron (1μM) and SDZ 205-557 (1μM) abolished responses to the 5-HT3 agonist, 2-Methyl 5-HT, confirming the efficacy of the antagonists. Conclusion: These results cast serious doubt regarding the role of endogenous serotonin acting on 5-HT3 and 5-HT4 receptors in the generation and propagation of colonic peristalsis. We suggest caution should be exercised when interpreting results of 5-HT antagonists on colon.

POS-WED-091

ANATOMICAL PROJECTIONS OF C3 ADRENERGIC NEURONS IN THE RAT MIDBRAIN, BRAINSTEM, AND SPINAL CORD

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Purpose: C1, C2, and C3 neurons constitute the three known adrenergic cell groups in the rat central nervous system. While these cell groups are known to be barosensitive, detailed analysis of efferent projection patterns is limited to C1 neurons. The aim of the current study is to detail the anatomical distribution of C3 efferents throughout the rat midbrain, brainstem and spinal cord through the use of a lentivirus which selectively expresses green fluorescent protein (GFP) in adrenergic and noradrenergic neurons via the PRSx8 promotor. **Methods:** The lentivirus was injected into the midline C3 cell cluster (n=2), located ventral to the fourth ventricle. After 5 weeks of recovery, rats were perfused transcardially, and their brains and spinal cords removed for processing. Sections were then processed via immunohistochemistry for GFP to identify regions containing C3 terminal fields. **Results:** All C3 terminal fields in the spinal cord were interacting with ChAT immunoreactive cell bodies or processes in the Intermediolateral Cell Column (C8-T13), as well as in Lamina 10, from upper cervical to sacral segments. In the brainstem, terminal fields were observed in commissural and medial nucleus of the solitary tract, caudal ventrolateral medulla, raphe pallidus, raphe obscurus, and the rostral ventrolateral medulla. Terminal fields within the pons were observed in the ventrolateral periaqueductal grey, the median and dorsal raphe nuclei, locus coeruleus, and A7 regions. **Conclusions:** By documenting the projections of C3 neurons throughout the central nervous system, we will gain a more comprehensive understanding of how these cells contribute to central cardiovascular circuitry.

POS-WED-092

LOCALIZATION OF OREXIN RECEPTOR 1 AND 2 IN THE ROSTRAL VENTROLATERAL MEDULLA OF RAT

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Orexin A and orexin B, two hypothalamic peptides, are important signalling molecules in feeding and sleep/wakefulness. Orexin A (OX-A) containing neurons, located exclusively in the lateral hypothalamus and perifornical area, project to several areas of the brain that are closely associated with cardiovascular regulation. The actions of this peptide are mediated by two G-protein coupled receptors, orexin receptor-1 (OX1R) and orexin receptor-2 (OX2R). Recent studies revealed the presence of OX-A immunoreactive fibres in the rat rostral ventrolateral medulla (RVLM). But the presence and extent of OX1R and OX2R and their co-localization with catecholaminergic neurons are not established. The aim of this study was to investigate the distribution of orexin A (OX-A) terminals, OX1R and OX2R in the rat RVLM and their apposition and co-localization with tyrosine hydroxylase (TH)-immunoreactive neurons in RVLM using immunohistochemistry. OX-A immunoreactive terminals were found throughout RVLM and made close appositions with a sub-population of neurons containing TH-immunoreactivity as well as TH-positive dendrites. RVLM also expressed a high level of OX1R and OX2R. In the RVLM, OX1R and OX2R were found in about 90% and 50% of TH-immunoreactive neurons, respectively. Furthermore, OX2R was prominent on terminals that closely apposed to TH-immunoreactive neurons and dendrites in the RVLM. These results indicate that the orexin system provides a link between the RVLM and other brain regions and play important roles in integrating the complex physiology underlying cardiovascular function.

POS-WED-093

IDENTIFICATION OF CYCLICAL PROPAGATING CONTRACTIONS ALONG ISOLATED FULL-LENGTH HUMAN COLON: ARE THERE DIFFERENCES IN SLOW TRANSIT CONSTIPATION ?

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Purpose: We characterized the motor activity along the intact full length human colon and determined whether differences exist in the colonic motility from patients with slow-transit constipation (STC). Methods: The entire colon (from cecum to rectum) was removed from 4 STC patients undergoing colorectal surgery, with prior written informed consent and the descending colon from 19 colorectal carcinoma patients. Following removal from the patient, the colonic preparation was mounted directly into an organ bath constantly bubbled with oxygenated Krebs' solution at 36oC. Isometric mechanical recordings were made from circular muscle simultaneously along the full length of each preparation. Results: In healthy segments of colon, propagating contractions were recorded from 7 of 19 intact preparations of descending colon, with a mean interval between contractions of 4.2 ± 0.7min, and mean amplitude and half duration of 14.4 ± 2.5g (n=7) and 54 ± 8.9s (n=7), respectively. In 3 of 4 whole colons removed from patients with STC, rhythmic propagating contractions were also recorded, that occurred every 6.9 min (P>0.05; n=3). In healthy colon, balloon distension at the oral end evoked a premature propagating contraction in 5 of 19 trials, which consisted of a descending wave of contraction preceded by a wave of descending inhibition. In 3 of 16 trials, at the anal end, evoked a local ascending contraction. Conclusion: These first recordings from isolated whole human colon reveal that propagating contractions can be recorded, which occur cyclically and propagate over large distances, consisting of a descending inhibitory phase. Propagating contractions can also be recorded from whole isolated colon in patients with STC.

POS-WED-094

THE EFFECTS OF PLANT-DERIVED ODOUR EXPOSURE ON THE ACUTE STRESS RESPONSE

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Purpose: Odorous exposure to isolated plant-derived chemicals has previously been demonstrated to have alleviating effects on the physiological responses to stress. However the effects of exposure to a novel combination of these chemicals has not been well characterised. The aim of this study was to assess the hormonal and general oxidative effects following an acute restraint stress with concurrent exposure to a novel combination of plant-derived odours, Praescent™ (α-pinene [0.015%], *cis*-3-hexen-1-ol [0.03%], and *trans*-2-hexenal [0.03%] diluted in triethyl citrate (vol/vol)). **Methods:** Male Wistar rats were exposed to no odour (n=14), triethyl citrate odour (n=10), Praescent™ odour (n=10), or 1% propionic acid odour (n=10) in the presence or absence of a concurrent two hour restraint stress. Blood samples were collected immediately following odour exposure and processed on ice. Plasma was used for analysis of corticosterone while phosphate-buffered saline washed erythrocytes were used as a 1% suspension in phosphate-buffered saline and a 10% distilled water hypolysate for general oxidative status and reduced glutathione determination respectively. **Results:** Acute restraint stress significantly increased plasma corticosterone concentration in animals exposed to no odour ($p<0.05$) and vehicle odour ($p<0.05$) while no significant increase was observed with exposure to Praescent™ or propionic acid odour. Exposure to Praescent™ odour was also able to prevent the stress-induced increase in general oxidative status ($p<0.05$) without affecting levels of reduced glutathione. **Conclusion:** Odorous exposure to the plant-derived chemical combination, Praescent™, can attenuate the increase in plasma concentrations of corticosterone and prevent the stress-induced increase in general oxidative status caused by acute restraint stress.

POS-WED-095

INTERLEUKIN-6 ACTIVATION OF MAP-KINASE PATHWAYS IN ADRENAL MEDULLARY CHROMAFFIN CELLS

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Purpose: The aim of this study was to characterize the action of interleukin-6 (IL-6) on the chromaffin cellas of the adrenal medulla. This is important because such an interaction may underlie the bi-directional relationship known to exist between the immune and neuroendocrine systems. Dysregulation of such an interaction may contribute to a number of pathologies, most notably those relating to stress. **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 salt solution and then incubated with IL-6 (1nM) for various periods of time. Standard immunoblotting and immunocytochemical procedures were used to detect activated proteins. All experiments were repeated at least three times using separate culture preparations. **Results:** IL-6 caused a transient increase in the phosphorylation of ERK1/2 but not p38 or JNK. Erk1/2 activation was maximal after 5-15 mins rising to approximately 150% of basal levels ($p<0.01$). While p38 and JNK were not responsive to IL-6 both became phosphorylated when the cells were incubated with histamine for 10 mins. As reported previously in addition to ERK1/2 activation IL-6 stimulated the tyrosine phosphorylation and nuclear localization of signal transducer and activator of transcription (STAT)3. This response was delayed compared to that of ERK1/2 rising to approximately 400% basal after 15 mins. **Conclusions:** These data provide evidence that the neuroendocrine chromaffin cells are sensitive to IL-6, suggesting a pathway linking immune-derived signals to the adrenal medullary stress response. This interaction results in the selective activation of the MAP-kinase pathway mediated by ERK1/2.

POS-WED-096

PROPAGATING MOTOR PATTERNS OF THE DIABETIC ILEUM

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The motility patterns of the diabetic small intestine and the pathophysiological mechanisms underlying altered intestinal transit in diabetes are not well understood. **Purpose**: To determine if propagating motor patterns are impaired in the ileum of diabetic C57Bl6-db/db mice and to determine if this coincides with loss of nitrergic nerves and/or interstitial cells of Cajal (ICC). **Method**s: The tunica muscularis of the most distal 1cm of ileum was processed for Kit, nNOS and tyrosine hydroxylase (TH) immunohistochemistry. The remaining ileum and mesentery was placed in a recording chamber, changes in ileal diameter were recorded via a video camera, and spatiotemporal D-maps constructed. Tension was recorded from proximal, mid and distal sites and extrinsic nerves stimulated via platinum wires positioned on the mesentery. **Results**: Two distinct patterns of motor activity were evident from D-maps and tension recordings of non-diabetic and diabetic ileum. In non-diabetic ileum, migrating motor contractions (MMCs), occurred at a frequency of 0.51±0.02min-1, propagated in a distal direction, and were superimposed on phasic contractions with a frequency of 29.3±0.8min-1 (n=15). Mesenteric stimulation evoked premature MMCs and increased MMC amplitude at proximal sites for 2-3 cycles post-stimulation (n=9). In diabetic ileum, MMCs were more frequent (0.63±0.03min-1) whilst phasic contraction frequency was reduced (25.9±1.2min-1;n=6). Responses to mesenteric stimulation were attenuated in diabetic ileum compared to non-diabetic controls. Kit-immunohistochemistry revealed a variable density of ICC between diabetic mice but there was no discernible difference in the distribution of nitrergic or sympathetic nerves between diabetic and non-diabetics. **Conclusions**: Changes in the characteristics of propagating contraction patterns in the ileum are likely to contribute to the altered intestinal transit commonly associated with diabetes. Loss of ICC-DMP, which are implicated as mediators of neurotransmission, may contribute to the attenuated responses of the diabetic ileum to extrinsic nerve stimulation.

POS-WED-097

ACTIVITY OF MECHANOSENSITIVE NEURONS INNERVATING THE URETHRO-GENITAL REGION OF FEMALE GUINEA-PIGS

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Purpose: To characterise sensory receptors innervating the female urethro-genital region, extracellular single unit recordings were made from the pudendal nerve during mechanical stretch or von Frey hair probing of urethral mucosa or serosa. **Methods:** Urethro-clitoris preparations, taken from humanely killed female guinea pigs (200-250g), were used to isolate single sensory fibres projecting to the urethro-genital region for electrical recording. The urethra was opened flat from the dorsal side with one edge attached to a hook for circumferential stretch of urethral wall and other edge fixed to the base of recording chamber with mucosa or serosa uppermost. **Results:** Spontaneous action potentials were observed in 3 out 8 experiments with frequency 20 ± 5 Hz (n=3). In some areas of the urethra, serosal probing with 1.5 mN von Frey hair evoked large action potentials with a firing frequency 11 ± 7 Hz (n=5). Similarly, mucosal probing in the area adjoining the caudal end of clitoris also evoked large action potentials with frequency 23 ± 7 Hz (n=3). Circumferential stretch of urethral wall with increasing force evoked large graded action potentials (frequency: 25 ± 12 Hz with 5g stretch; 35 ± 13 Hz with 10g stretch; 45 ± 34 Hz with 20g stretch). **Conclusions:** These data indicate that pudendal stretch-sensitive mechanoreceptors can be activated by mucosal and serosal probing of urethral wall. A high density of mechanosensitive endings was found in the junction area between urethra and caudal end of clitoris.

POSWED-098

IDENTIFICATION OF A NOVEL ROLE OF LAMININ BETA1 IN IMPAIRING SPATIAL LEARNING IN RATS

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Extracellular matrix proteins are necessary for neuronal migration, neurite outgrowth, growth cone guidance, synapse formation and stabilization. Along with collagen, laminins are the major structure elements of the basal lamina. The role of laminin is widely studied in the neuromuscular junction. In rodents, laminin is highly expressed in hippocampal neuronal cell layers, but little is known regarding the role of laminin β1 in neuronal plasticity. In the present study, we found that rats subjected to water maze training showed a reduced level of laminin β1 mRNA and laminin β1 protein expression in hippocampal CA1 area. The expression level of laminin β2 was not affected. When we transfected the Flag-laminin β1 plasmid to rat CA1 area, it significantly impaired spatial learning. Rats also spent less time in the target quadrant for the probe trial test. On the other hand, transfection of laminin β1 siRNA to CA1 area to knock down endogenous laminin β1 expression facilitates spatial learning. Rats also spent more time in the target quadrant for the probe trial test. In studying the laminin β1 signaling pathway, we found that laminin β1 siRNA transfection increased the phosphorylation level of ERK1/2 and protein kinase SGK at Ser-422. The phosphorylation level of AKT at Ser-473 was not changed. But over-expression of laminin β1 produced the opposite effect. Further, inhibition of ERK1/2 decreased SGK phosphorylation at Ser-422. These results suggest that laminin β1 impairs spatial learning through inhibition of ERK1/2 -- SGK signaling in rat hippocampus.

POS-WED-099

NEURONAL ACTIVITY IN A POSTERIOR PARIETAL CORTICAL AREA (LIP) IN THE MACAQUE CORRELATE WITH PERFORMANCE IN AN ATTENTION TASK

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Purpose: We have observed an information-processing bottleneck in an attentional task in macaques. We investigated whether the neural activity in the lateral intraparietal area (LIP), a part of the posterior parietal cortex that is believed to control attentional priorities, was correlated with the monkey's behaviour in the above task. **Methods:** Two macaque monkeys were trained on a delayed match to sample memory task, where they had to match both the location and orientation of two grating patches that were presented successively with a variable delay between them (up to 600 msec). Recordings were made from LIP in one monkey during this task while a measure of sensitivity (d') was calculated from his behavioural responses. Since the relationship between d' and the delay was variable between days, we tested whether changes in d' covaried with a neuronal metric in LIP. **Results:** While variation of the delay between the two gratings led to a function generally similar to the classical attentional blink (AB) in humans, showing poorer performance for delays between 50 and 450 msec, there was also variation in this function from day to day. Recordings made from 23 LIP neurones showed that the ratio of the responses to the two (optimal) grating patches followed the variations in d' ($r = 0.32$, $p<0.001$, $n=98$). For a subset of cells (12/23) that showed especially close correlation ($p<0.1$), the relationship was very strong ($r=0.79$, $p<0.001$, $n=53$). **Conclusions:** Our results provide the first direct single neuron correlate of an attentional overload similar to the 'attentional blink' observed in humans. While imaging studies have implicated several cortical sites for the AB in humans, our results show at the single cell level that the pattern of activity in LIP shows a high degree of correlation with the changes in performance (d') as a function of the delay between stimuli. This is consistent with the idea that LIP is the primary site of the attentional overload.

POS-WED-100

SEX-SPECIFIC PATTERNS OF DNA METHYLATION WITHIN THE VENTROMEDIAL PREFRONTAL CORTEX AS A PUTATIVE MECHANISM FOR SEX DIFFERENCES IN FEAR-RELATED LEARNING AND MEMORY

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Epigenetic mechanisms support learning and memory. In this study we explored whether sex differences in fear extinction memory are associated sex-specific patterns of DNA methylation within the ventromedial prefrontal cortex. In female C57/Bl6 mice, fear extinction memory was impaired relative to male mice, and female mice showed signs of greater fear incubation. Purpose: As DNA methylation has previously been shown to regulate gene expression during the acquisition of conditioned fear, we went on to determine whether these sex-specific behavioural effects are associated with differences in the distribution of this epigenetic modification throughout the genome. Methods: Genome-wide DNA methylation profiling (methyl binding domain (MBD)-mediated enrichment followed by microarray analysis) was performed on tissue derived from the ventromedial prefrontal cortex; vmPFC, a brain region known to be involved in emotional learning and memory. Results: A number of gene promoters showed a greater than 2-fold difference in methylation between male and female naïve mice (n=7 per group). For example, the promoter region of Rab3a (a synaptic plasticity-related gene) was identified as being highly methylated in females but not in males. qPCR analysis revealed that this pattern of DNA methylation correlated with decreased Rab3a mRNA expression in female mice. Conclusion: These data imply that epigenetic modifications such as DNA methylation may play an important role in sex-specific biases in relation to fear-related memory. Currently, we are extending our genome-wide approach to determine whether fear and extinction learning are associated with dynamic sex-specific epigenetic regulatory processes.

POS-WED-101

ADULT CANINE NEUROGENESIS: EVIDENCE FOR A DORSAL-VENTRAL GRADIENT BASED ON HISTOLOGY AND *IN VITRO* NEUROPRECURSOR ASSAYS

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PURPOSE: Neurogenesis has been observed in the hippocampus of numerous adult mammalian species as assessed by immunohistochemistry (IHC) and *in vitro* assays of stem cell activity. The canine is unique as it contains two distinct dentate gyral areas in the dorsal and ventral positions. This study aims to assess whether there is a neurogenic gradient between the two regions of the adult canine hippocampus. **METHODS:** Whole hemisphere coronal sections of adult canine brain were immunohistochemically examined for Doublecortin (DCX). Dorsal and ventral hippocampal regions were also dissected from post mortem canine brain, with cells dissociated and transferred to bulk neurosphere culture or colony forming assay to quantify proliferation potential. Polymerase Chain Reaction (PCR) analysis and EDU proliferation studies were also performed on early passage cells. **RESULTS:** The dorsal region of the canine hippocampus had a much higher density of DCX positive cells than the ventral region. Cells from both regions were capable of proliferating to form neurospheres *in vitro* after primary passage, and expressed neuroprecursor markers on PCR and immunocytochemical analysis. The colony forming assay (n=3) revealed a significantly larger number of spheres > 10um in the dorsal hippocampus ($p<0.05$), while EDU proliferation studies also support a dorsal-ventral gradient. **CONCLUSIONS:** Combined histological and *in vitro* analyses suggest an increased level of neurogenesis in the dorsal part of the canine hippocampus compared to ventral regions.

POS-WED-102

RIGHT FRONTAL THETA ACTIVATION LINKED TO AVERSION AND NEUROTICISM

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Purpose: Approach-avoidance conflict is thought to generate negative affective bias, distinct from that elicited by simple aversive input. Using an economic paradigm, we varied the value of gain-loss combinations (gain held constant with increasing loss value) so that conflict and simple aversive activations should peak in different conditions. We then tested for simple aversive- and conflict-related activations of theta rhythmicity. **Results:** At both frontal midline and right frontal sites, theta activation increased as loss increased in both females (N=13) and males (N=15). Increased right frontal loss activation was significantly correlated with increased behavioral aversion and neuroticism; with a major contribution to these effects from female participants. Right frontal theta activation appeared greater with conflict than loss but this difference only approached statistical significance. In contrast, the midline theta activation showed no sign of sensitivity to conflict. **Conclusion:** Right frontal theta activation, which could include a conflict component, is involved in 'what not to do' processing. Individual differences in right frontal neural response could potentially be used as a marker of behavioral and emotional reactivity to aversive events.

POS-WED-103

FUNCTION OF EGF RECEPTOR SIGNALING IN THE MODEL OF SCHIZOPHRENIA

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There are EGF and EGF receptor (ErbB1) abnormalities in brain tissues and blood of schizophrenic patients. Here we examined pathological roles of ErbB1 signaling in the schizophrenia model rats that are established with bilateral microinjections of ibotenic acid to the ventral hippocampus as neonates. Treatment with ibotenic acid altered HB-EGF and TGF alpha protein levels in the forebrain regions in parallel with persistent activation of ErbB1 receptors. On postnatal week 8, rats were tested for behavioral tasks. The hippocampus-lesion significantly impaired the behavioral scores of PPI and latent learning in adults. In context fear conditioned task, memory retentions were not affected although their latent learning was significantly disrupted. We determined the antipsychotic effects of several ErbB1 tyrosine kinase receptor inhibitors in this model. The abnormalities in PPI and latent inhibition were ameliorated by icv infusion of all the ErbB1 inhibitors from an osmotic pump. These results indicate that blockade of ErbB1 signals may be a novel antipsychotic target for schizophrenia and its related disorders.

POS-WED-104

PERCEPTUAL RIVALRY IS GRADUAL

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Purpose: In rivalry, constant physical stimuli evoke distinct perceptual interpretations (percepts) that fluctuate between being dominant or suppressed over time. For any given region of visual space, distinct percepts are usually characterized as mutually exclusive, although different spatial regions may belong to different percepts (piecemeal rivalry). Here we investigate whether rivalry is an all-or-none or a gradual process. **Methods:** First, we present two orthogonal gratings, that differ in their luminance, contrast, or movement direction, to each eye separately, thereby inducing binocular rivalry (n=10). Second, we use two reflexes, pupil dilation and the gain of the optokinetic nystagmus (OKN), as objective measures of dominance during rivalry. Third, we use an analog joystick as continuous input device for a gradual subjective report. **Results:** Both pupil size and speed of OKN's slow phase show that transitions between percepts are smooth and gradual. By simulating wave-like transitions between percepts, we find that piecemeal rivalry largely explains the gradual nature of transitions. Similarly, observers' joystick deflections, which are highly correlated with the reflex measures, indicate gradual transitions. In addition, the objective measures allow assessing rivalry without active report and reveal a significant effect of response mode (none, button press, joystick) on rivalry dynamics. This suggests that the previously described global all-or-none nature of rivalry may be a consequence of a discrete set of possible responses, shadowing gradual transitions between percepts. **Conclusion:** Thus, rivalry is a gradual phenomenon on a global scale, but likely discrete for any given point in visual space.

POS-WED-105

APPETITIVE TO AVERSIVE COUNTERCONDTIONING

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Purpose: Appetitive and aversive motivational states are frequently viewed as independent and mutually inhibitory. We examined this using appetitive to aversive counterconditioning, where fear learning to a previously established appetitive CS is retarded relative to a novel control. **Methods:** Rats (in each group, n = 8) underwent Pavlovian appetitive to aversive counterconditioning. Experiment 2 studied whether this effect was dependent on appetitive properties of the CS or if it could be attributed to the history of CS exposure per se. Thus an additional control was included, involving unpaired presentations of the CS and reward. Experiments 3 and 4 also examined differences in attention allocated to the CS through manipulation in the magnitude of the outcome. **Results:** Experiment 1 replicated counterconditioning as demonstrated by Bouton and Peck (1992). Experiment 2 demonstrated retardation of freezing during Stage II aversive conditioning for both groups who had CS exposure during Stage I relative to the control. However these groups differed on expression of the appetitive CR. Experiments 3 and 4 demonstrated that changes in surprise does not restore associability of the CS and thus did not ameliorate the retardation of fear learning. **Conclusions:** Taken together, these results suggest that manipulations which restore the associability of an appetitive CS do not overcome the retardation of fear learning during appetitive to aversive counterconditioning. They are consistent with the possibility that retardation is due to motivational competition between appetitive and aversive systems.

POS-WED-106

TRANSPLANTATION OF STEM CELLS INTO THE YOUNG IRRADIATED MOUSE BRAIN

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Stem cells reside in important areas involved in cognition in the brain and these areas are severely injured by irradiation. We have previously demonstrated that voluntary exercise in mice is able to rescue endogenous stem cells, neurogenesis levels and alter the structural integration of immature neurons in the hippocampus that are injured after irradiation. We irradiated mice (n=10) with a moderate dose of irradiation (8 Gy) at postnatal day (P) 9 and injected 1.0 x 10^5 syngenic mouse stem cells into the hippocampus at P22. Animals were then given free access to running wheels and were sacrificed after 30 days. There was no difference between the level of activity in the SHAM-running and irradiated-running mice, indicating a comparable level of physical activity. There was no significant difference ($P>0.05$) between SHAM or irradiated animals in the survival of BrdU+ transplanted stem cells. However, after irradiation, 50% of the injected stem cells in non-running animals were undifferentiated or showed an increased fate differentiation towards a glial lineage, a significant difference compared to SHAM mice. These results indicate transplanted stem cells into the irradiated hippocampus are pushed significantly towards a glial instead of neuronal fate. In addition, there is a significantly perturbed differentiation of early-stage neurons (Doublecortin+ cells) in irradiated animals ($P<0.05$). Preliminary results indicate that injection of stem cells after irradiation in combination with running enhances neuronal lineage differentiation. If we can mitigate the adverse side effects of radiotherapy in the increasing number of survivors of childhood cancer, we would improve their quality of life.

POS-WED-107

THE EFFECT OF FOOTSHOCK AND RESTRAINT STRESS ON TYROSINE HYDROXYLASE PHOSPHORYLATION

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Tyrosine hydroxylase (TH) is regulated acutely by protein phosphorylation and chronically by protein synthesis. In these studies we aimed to investigate TH phosphorylation in the rat locus coeruleus (LC) and the adrenal medulla (AM) within the first 40 min of exposure to either footshock or restraint stress. **Methods:** Rats were exposed to footshock (FS+) or restraint (R+) stress for 10, 20 or 40min. Sham controls (FS- and R-) were exposed only to the novel environment and not to the stressor, while home cage controls were used for basal measurements (n=6/group). Animals were sacrificed immediately after the stress protocols and TH phosphorylation in the LC and AM was analysed using western blotting. **Results:** Ser40 phosphorylation was significantly increased in the FS- relative to the FS+ rats at 40min only in the LC. Ser31 phosphorylation was significantly increased in the FS+ relative FS- rats at 10, 20 and 40min in the AM and at 20 and 40 min in the LC. Ser31 phosphorylation was also significantly increased in the R+ relative to R- rats at 10min in the LC and at 20 min in the AM. Ser19 phosphorylation was significantly increased in the FS- relative to FS+ rats at 10 and 20 min in the AM. **Conclusion:** TH phosphorylation at Ser40 and Ser19 was increased in response to novelty, but not to footshock and restraint stress, in either the LC or the AM; while TH phosphorylation at Ser31 was increased in response to footshock and restraint stress in both the LC and the AM.

POS-WED-108

MILDRONATE, A SMALL MOLECULE, ENHANCES MEMORY AND ADULT HIPPOCAMPAL NEUROGENESIS IN RATS

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Adult neurogenesis is shown to be impaired in diseases manifested as neurodeficiency/memory loss. **Purpose:** We suggest that mildronate (small molecule of beta-butyrobetaine class) which previously demonstrated neuroprotection in different neurotoxicity models (Pupure et al., 2010; Klusa et al., *in press*), is capable to influence the memory processes and regulate adult hippocampal neurogenesis. **Methods:** Male Wistar rats were observed in conditioned avoidance (CAR) and passive avoidance response (PAR) tests; additionally, scopolamine-amnesia model was used. Mildronate was administered intraperitoneally at 10, 20 and 50 mg/kg for two weeks. In CAR test, on the day 8 of mildronate administration, rats received BrdU (75 mg/kg i.p., 7 days), combined by CAR training sessions. Double immunohistochemical and triple immunofluorescent methods were used to assess BrdU incorporation within the dividing cell nuclei (DAPI for nuclear counterstain), and nestin-positive cells in rat hippocampal gyrus dentatus. **Results:** In PAR test, mildronate (20, 50 mg/kg) increased the step-through latency ($p=0.02$ and $p=0.003$, resp.), and at 50 mg/kg reversed the scopolamine-induced amnesia ($p<0.05$). In control group (saline + BrdU), a majority of neurons expressed nestin; while BrdU and BrdU/nestin co-staining was negligible. CAR training significantly increased the number of BrdU/nestin co-stained neurons and decreased nestin expression. Mildronate + BrdU group showed a considerable increase in the number of BrdU/nestin co-stained cells. Moreover, in mildronate + BrdU + CAR group, the BrdU/nestin co-staining was increased about 4-fold vs. control values. Similar results were obtained by immunofluorescence. **Conclusion:** Mildronate stimulates learning/memory processes, and enhances adult hippocampal neurogenesis, indicating mildronate as therapeutic candidate in the treatment of neurodegeneration-related cognitive decline. Acknowledgements: ESF Nr.2009/0217/1DP/1.1.1.2.0/09/APIA/VIAA/031.

POS-WED-109

REDUCED HIPPOCAMPAL NEUROGENESIS IN AMES DWARF MICE

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Purpose: Hippocampal neurogenesis occurs throughout adult life in mammals but declines with ageing. Decreased neurogenesis may contribute to age-related cognitive deficits. One approach for identifying key elements influencing neurogenesis in ageing is to study the neurogenic process in long-lived mouse mutants. The Ames dwarf mouse has an extended lifespan, living approximately 50% longer than their wild-type siblings. Hippocampal neurogenesis has been reported to be more than doubled in 3 month old dwarf mice compared to age-matched normal siblings, and memory retention is maintained with age in dwarf mice. The aim of this study was to determine whether there are any differences in neurogenesis in aged Ames mice compared to age-matched control littermates. **Methods:** Aged (22 months old) homozygote Ames dwarf mice and age-matched littermate controls (heterozygote/wildtype) (n=9 per group) were subjected to open-field testing and the novel object recognition test. Following behavioural testing, mice were given BrdU (200mg/kg i.p. for 2 days) before the brains were taken for immunohistochemical analysis 4 weeks later. **Results:** There were no differences in locomotor activity or anxiety levels between dwarf mice and controls in an open field. Dwarf mice showed increased exploration towards a novel object compared to controls suggesting better recognition memory. Quantitative stereological analysis showed no difference in numbers of Ki67-immunoreactive cells at the hippocampal subgranular zone between the two groups. In contrast, numbers of doublecortin-immunoreactive neurons were significantly reduced in dwarf mice by 46% compared to littermate controls ($P<0.02$, Students t-test). Similarly, we found BrdU-positive cells were more abundant in the controls compared to dwarf mice. **Conclusions:** These results suggest that new hippocampal neurons do not participate in the formation of recognition memory.

POS-WED-110

GENDER DIFFERENCES IN CYTOGENESIS AND BEHAVIOR AFTER IRRADIATION TO THE DEVELOPING MOUSE BRAIN

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Radiation therapy to the brain used for the treatment of pediatric malignancies may lead to difficulties in learning and memory later in life. These late effects may be influenced by age, treatment and gender, where young girls suffer more severe late effects compared to young boys. Unfortunately, most studies are performed on male animals, and no consideration of gender is taken. Therefore, we wanted to investigate possible long-term gender differences in the response to irradiation (IR). Mice of both genders were irradiated with a single dose of 8 Gray to the whole brain on postnatal day 14. Learning was evaluated using the IntelliCage platform when mice reached adulthood (3 months). We found a significant difference in reversal learning where irradiated male, but not female, mice performed worse than controls. To investigate gender differences in hippocampal cytogenesis, mice were injected with bromodeoxyuridine (BrdU) four weeks prior to euthanasia. The brains were analyzed by counting BrdU-positive cells in the hippocampal dentate gyrus (DG), known to be radiosensitive. This revealed a significant gender difference in the granule cell layer, where the density of BrdU-positive cells after irradiation decreased more in female compared to male mice. Proliferation in the DG was investigated by counting phospho-histone H3-positive cells 6 hours and 14 weeks after IR. There was a significant loss of proliferating cells in the subgranular zone (SGZ) in the DG at both time points. In the adult, non-irradiated brains, females showed 16% more proliferating cells in the SGZ compared to males. In summary, our results indicate that gender affects the outcome, both in behavior and cytogenesis after IR to the developing mouse brain.

POS-WED-111

BRAIN-DERIVED NEUROTROPHIC FACTOR VAL66MET POLYMORPHISM INFLUENCES THE MAGNITUDE OF HUMAN LONG-TERM POTENTIATION WHICH PREDICTS MEMORY PERFORMANCE

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Brain-derived neurotrophic factor (BDNF) has been identified as an important modulator of synaptic plasticity in animals and humans. A single nucleotide polymorphism in the BDNF gene resulting in a valine-to-methionine substitution at codon 66 (val66met) has been shown to affect regulation of BDNF and is associated with poorer memory. It has been hypothesized that this polymorphism affects long-term potentiation (LTP) in humans, but this has not been confirmed due to difficulties in measuring LTP in humans. **Purpose:** The present study tested whether the BDNF val66met polymorphism was associated with LTP in humans. We also tested whether LTP and BDNF val66met polymorphism was predictive of memory. **Methods:** 20 healthy participants (10 females) were recruited. Blood was taken and DNA samples were extracted of the 113 base-pair polymorphic BDNF section, with participants defined as ValVal, Val/Met or Met/Met. EEG was used to record changes in amplitude of visual evoked potentials (VEP), and the WMS-III was used to measure visual memory. **Results:** Val/Val variants had significantly higher VEP amplitude increase compared to individuals with a Met allele ($p<.05$). Val/Val variants also performed significantly better on measures of visual memory ($p<.05$). The degree of LTP also significantly predicted memory score ($p<.05$). **Conclusions:** This study provides evidence that BDNF affects LTP in humans, as well as the first functional evidence of LTP in humans.

POS-WED-112

SPATIAL WORKING MEMORY LEARNING CAN BE REPEATEDLY TESTED AND CHALLENGED WITH ANTERIOR THALAMIC LESIONS

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Purpose: Memory deficits occur in many brain disorders, including stroke and dementia. Anterior thalamic (ATN) damage produces dense amnesia in humans and loss of spatial and temporal order memory in rats. **Method:** We tested Adult male Long Evans rats in a T-maze (with varying start arm positions) on spatial non-matching to sample before and after ATN lesions and then, in separate groups, after either a 1-week (N=19) or 4-month (N=20) break. Lesions were made by 3 injections of N-methyl-D-aspartic acid into the ATN on each side. Sham controls received the same surgery without fluid injection. **Results:** All rats reached ~85% correct initially. Rats with ATN lesions dropped to chance levels throughout post-operative testing. Interestingly, sham rats performed at lesion levels at the start of both post-operative tests with both break lengths but relearned to 85%. Thus a delay as short as one week produces forgetting of the task, when 24 hours does not. **Conclusion:** This shows that the effects of lesions on learning (and not just memory) can be repeatedly tested with brief periods of testing provided a break of at least a week is inserted between tests. This confirms and extends a previous report of partial relearning by shams against a background of no learning in ATN lesion damage in the same task.

POS-WED-113

ICONIC MEMORY CAN IMPROVE DECISION ACCURACY

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Purpose: Simple decisions about features or properties of visual stimuli have been shown to involve a process of accumulating noisy sensory information over time, until a criterion amount of information has been attained and a decision can be made. High-resolution sensory information (iconic memory) is accessible for several hundred milliseconds following stimulus offset. The aim of the current study was to determine whether accumulation of evidence could continue in the absence of the physical stimulus via representation in a memory store. **Methods:** Six participants were asked to judge whether the coherent motion in a random-dot stimulus was moving left or right (2AFC). Participants responded either immediately (speed accuracy trade-off), after a varying blank delay (100-800ms), or after a masked delay (100-800ms). **Results:** This study found that by simply delaying post-stimulus responses while viewing a blank screen, participants show improved decision accuracy for up to 400ms. However, when the blank screen was replaced by a sensory masking stimulus, which has been shown to interfere with iconic memory, accuracy was significantly lower. **Conclusion:** The results of this study suggest that we can continue to accumulate evidence from an iconic store in the absence of any physical stimulus and hence improve decision accuracy.

POS-WED-114

FROM LAPSE TO RELAPSE: BEHAVIOURAL MECHANISMS FOR RE-ACQUISITION OF EXTINGUISHED DRUG-SEEKING

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Purpose: Relapse to drug taking after a period of abstinence is typically florid and invloves a rapid return to pre-abstinence levels of drug intake. We developed an animal model of this rapid re-acquisition and studied the role of context in determining the rate of re-acquisition of extinguished reward-seeking. **Methods:** Rats (in each group, n = 8) were trained in an instrumental paradigm to respond for alcoholic beer on an FR-1 schedule. They were then extinguished in a familiar context, novel context, or remained in the home-cage. Rats were then re-trained on an FR-1 schedule or tested using a progressive ratio schedule. **Results:** Re-acquisition during re-training was faster than acquisition regardless of whether re-training occurred in the original training context, the extinction context, a novel context, or a context with a mixed history of reinforcement. Nonetheless, context did significantly influence re-acquisition via affecting latency to the first response: rats took significiantly longer to initiate responding when tested in the extinction context. **Conclusion:** These results are consistent with the suggestion that re-acquisition of drug and reward seeking is determined by an inhibitory influence caused by the extinction context and a facilitatory influence caused by reintroduction of the reinforcer (Bouton, 1993).

POS-WED-115

LOCALISATION OF THE CREATINE TRANSPORTER IN THE HUMAN BASAL GANGLIA

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Purpose: Creatine is an endogenous molecule taken up by the creatine transporter (CrT) to be used by the brain to improve energy metabolism. It is in clinical trials for Huntington's (HD) and Parkinson's disease (PD). Despite the progress of these trials, the distribution of the CrT has not yet been investigated in the human brain. **Methods:** We have used immunohistochemical techniques on free-floating neurologically-normal human brain sections (n=6) using a rabbit anti-CrT antibody to determine the localisation of the CrT in the basal ganglia. **Results:** Single and double labelling shows that the CrT is found within specific neuronal populations in the basal ganglia and not in glial cells. CrT immunoreactivity (IR) is found mainly within large projection neurons of the globus pallidus, subthalamic nucleus and substantia nigra pars reticulata. There was no CrT-IR within the projection neurons of the striatum or substantia nigra pars compacta. These results show that CrT-IR is present in subsets of neurons which are spared in HD and PD but show a low to undetectable IR within neurons which specifically degenerate. These findings suggest that the medium spiny neurons and the dopaminergic pars compacta neurons, which degenerate in HD and PD, have a poor Cr uptake capacity whereas cells which do not degenerate are capable of taking up Cr. **Conclusion:** Creatine now being trialled for HD and PD may potentially be exerting its effect on a broader population of cells in the brain than those primarily affected in these diseases.

POS-WED-116

INFLAMMATION AFTER AXONAL INJURY IN THE RAT CNS - MACROPHAGES AT THE INJURY SITE EXHIBIT DIFFERENT PHENOTYPES

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Introduction. In the injured mammalian CNS, macrophages have been implicated in both exacerbating and ameliorating damage to tissue. Previous work in injured mouse striatum demonstrated axons sprouting in close physical proximity to subsets of wound macrophages. The fibres course towards the wound edge, forming varicosities with microglia along the way. These sprouting fibres terminate in dense plexuses, within which lie macrophages. The fibres do not extend into the wound cavity, where macrophages of apparently identical morphology and immunoreactivity are found less than 100µm away. **Hypothesis.** At least two different macrophage phenotypes exist within the injured CNS - a cytotoxic macrophage capable of causing cell death in the core of the wound, and a reparative macrophage capable of stimulating wound repair at the wound edge. **Aims.**The aims of this study are to (1) verify that different macrophage phenotypes exist after injury to the CNS in the rat, and (2) develop immunohistochemical markers of the different macrophage phenotypes.**Methods.** Adult male rats were given bilateral brain axonal injury using a Scouten wire-knife. They were sacrificed at two weeks after injury was and brains were processed for immunohistochemistry. Ox42-positive macrophages (3000 edge, 2100 core) were pooled (n=3) and laser-catapulted into Trizol. RNA was extracted and purified with a Qiagen RNeasy kit. cDNA was prepared using the Invitrogen Superscript III Amplification Kit. Gene expression profiles of 30000 genes were compared on Affymetrix Rat Genome 230 2.0 Array. **Results.** Macrophages from the two locations differentially expressed 928 genes at least two-fold. Potentially targetable families of genes included transcription factors and regulators of axonal growth. **Conclusion.**The macrophages from two different locations at the wound exhibited differential gene expression, even though they were isolated from locations less than 100µm apart. We have shortlisted possible immunohistochemical markers that may identify, and differentiate between the supportive and non-supportive macrophages.

POS-WED-117

POLYMORPHISMS IN THE RECEPTOR TYROSINE KINASE MERTK GENE ARE ASSOCIATED WITH MULTIPLE SCLEROSIS SUSCEPTIBILITY

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Multiple sclerosis (MS) is a debilitating, chronic demyelinating disease of the central nervous system affecting over 2 million people worldwide. The TAM family of receptor tyrosine kinases (Tyro3, Axl and MerTK) have been implicated as important players during demyelination in both animal models of MS and in the human disease. We therefore conducted an association study to identify single nucleotide polymorphisms (SNPs) within genes encoding the TAM receptors and their ligands associated with MS. Analysis of genotype data from a genome-wide association study which consisted of 1618 MS cases and 3413 healthy controls conducted by the Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) revealed several SNPs within the *MERTK* gene (Entrez Gene ID: 10461) that showed suggestive association with MS. We therefore interrogated 28 SNPs in *MERTK* in an independent replication cohort of 1140 MS cases and 1140 healthy controls. We found 13 SNPs that replicated, with 7 SNPs showing p-values of $<10^{-5}$ when the discovery and replication cohorts were combined. All 13 replicated SNPs were in strong linkage disequilibrium with each other. In combination, these data suggest the *MERTK* gene is a novel risk gene for MS susceptibility.

POS-WED-118

CREB1 AND CREB BINDING PROTEIN IN STRIATAL MEDIUM SPINY NEURONS REGULATE BEHAVIOURAL RESPONSES TO PSYCHOSTIMULANTS

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Purpose: cAMP responsive element binding protein 1 (CREB1) has a complex influence on behavioural responses to drugs of abuse. The purpose of this study was to investigate the role of striatal CREB1 in behavioural responses to psychostimulants. **Methods:** Using the cre/lox recombination system we generated mice with a postnatal deletion of CREB1 directed to striatal medium spiny neurons. qRT-PCR and immunohistochemistry confirmed the CREB1 deletion. $CREB1^{DARPP\text{-}32Cre/loxlox}$ mice and littermate controls were assessed with respect to their acute (20mg/kg, n = 32 WT, 14 KO), and sensitized (10mg/kg, n = 21 WT, 14 KO) locomotor response to cocaine; amphetamine-induced stereotypies (10mg/kg, n = 5 WT, 7 KO) and ethanol hypnosis (3.5g/kg, n = 9 WT, 9 KO). We also generated mice with a striatal deletion of CREB binding protein (CBP), and assessed their response to acute cocaine (20mg/kg, n = 11 WT, 13 KO) or amphetamine (10mg/kg, n = 5 WT, 6 KO). **Results:** $CREB^{DARPP\text{-}32Cre/loxlox}$ mice show increased expression of sensitization (p = 0.012) and increased amphetamine-induced stereotypies (p = 0.023) compared to controls. $CBP^{DARPP\text{-}32Cre/loxlox}$ mice had a more pronounced phenotype, with an enhanced psychomotor response to acute cocaine (p = 0.016) and amphetamine (p < 0.001).

POS-WED-119

CLINICAL EVALUATION OF OXIDATIVE STRESS AND ANTIOXIDANT POTENTIALS IN PARKINSON PATIENTS

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Purpose: Recent studies have increasingly shown that oxidative stress has an important role in neurodegenerative diseases. However, investigating the oxidative stress or the antioxidant potentials are difficult to measure in a clinical practice. In this study, we measured serum levels of them by using with a simplified measuring system in Parkinson patients. **Methods**: We recruited control subjects without any abnormal neurological condition and outcome patients, including Parkinson's disease (PD), Alzheimer's disease (AD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and cerebrovascular disease (CVD). Blood samples were obtained from subcutaneous veins of upper extremities with a usual procedure. Oxidative stress and antioxidant potentials were analyzed by d-reactive oxygen metabolites (d-ROMs) test and biological antioxidant potential (BAP) test using with FRAS4 (Diacron International, Grossete, Italy). All participants were divided into 4 matrix subgroups at 300 U.CARR (Carratelli Unit) of d-ROMs test and 2200 μmol/l of BAP test. Statistical analysis was performed among controls, Parkinsons and other neurological disorders. **Results**: We could recruit 481 participants (female; 230, mean age; 70.0), including 61 controls, 107 PD patients, 12 AD patients, 18 MSA patients, 12 PSP patients and 253 CVD patients. Mean d-ROMs and BAP were 324.6 and 2537 in PD, and 345.1 and 2473 in all participants. These results were not significantly different from controls or other neurodegenerative disorders, but d-ROMs were lower and BAP was higher than CVD patients. In matrix analysis, there was no difference between PD and controls. CVD patients showed higher oxidative stress and lower antioxidant potential. **Conclusion**: We could evaluate serum level of oxidative stress and antioxidant potentials in various neurological disorders. These results suggested that serum level of them could reflect systemic oxidative stress, such as atherosclerosis or muscle fatigue, rather than that in the brain induced with neurodegenerative process.

POS-WED-120

SUB-ACUTE LPS EXPOSURE MODULATES THE NEURAL MICROGLIAL REACTION TO ACUTE ASPHYXIA IN PRETERM FETAL SHEEP

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Purpose: There is increasing evidence that exposure to lipopolysaccharide (LPS), a bacterial cell wall component, can sensitise (precondition) the brain to subsequent hypoxic-ischemic damage, and thus could exacerbate brain injury after preterm birth. However, the effects of prolonged exposure are unclear. In this study we examined the hypothesis that subacute exposure to LPS would potentiate neural inflammation after subsequent exposure to severe asphyxia. **Methods**: Chronically instrumented 103 day old (0.7 gestation age: term 147 days) fetal sheep in utero were randomized to 4 groups (n=8, all groups): saline infusion plus sham umbilical cord occlusion (saline-sham); saline infusion plus asphyxia induced by umbilical cord occlusion for 15 min (saline-occlusion); LPS infusion plus sham occlusion (LPS-sham); LPS infusion plus umbilical cord occlusion for 15 min (LPS-occlusion). Fetuses received either LPS as a continuous low dose infusion (100 ng over 24h, followed by 250 ng/24h for 4 days) plus boluses of 1 μg LPS at 48 h, 72 h and 96 h, or the same volume of normal saline. Asphyxia or sham occlusion was induced on day 5, and fetuses were euthanized 5 days after occlusion. Counts of immunohistologically labelled cells were undertaken on multiple sections in the peri-ventricular white matter using the optical fractionator probe technique. **Results**: LPS infusion was associated with a significant increase in microglia, astrocytes and caspase-3 +ve cells in periventricular white matter compared to saline-sham (P<0.01). Umbilical cord occlusion was associated with a significant increase in reactive microglia, astrocytes and caspase-3 +ve cells compared to saline-sham and LPS-sham controls (P<0.01). LPS-occlusion was associated with a significant reduction in numbers of reactive microglia (P=0.01) and caspase-3 +ve cells compared to saline-occlusion (P=0.002). Although there was no significant change in numbers of CNPase +ve cells after either LPS-occlusion or saline-occlusion, activated caspase-3 co-localised with CNPase. **Conclusions**: In preterm fetal sheep, sub-acute exposure to LPS over 5 days attenuates the inflammatory reaction and reduces apoptosis in white matter after subsequent acute severe asphyxia.

POS-WED-121

EXCITATORY EFFECTS OF L-DOPA ON NIGRAL DOPAMINERGIC NEURONS IN BRAIN SLICES

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L-DOPA has been shown to induce oxidative stress and death of cultured dopaminergic neurons. These cell culture studies have raised concerns as to whether similar toxic mechanisms contribute to the side effects and the continued degeneration of nigrostriatal dopaminergic neurons during treatment of Parkinson's disease. **Purpose**: Our aim was to investigate unconventional effects of L-DOPA using brain slice model, focusing on possible excitotoxic effects. **Methods**: Conventional extracellular recordings were made from nigral dopaminergic neurons in acute rat midbrain slices. **Results**: L-DOPA (0.05, 0.3, 1 and 3 mM) produced a dose-dependent inhibition of firing of nigral dopaminergic neurons. Sulpiride (D2 receptor antagonist) reversed this inhibition, and unmasked an excitatory effect. Excitation was potentiated by L-DOPA auto-oxidation (n=10), and attenuated by AMPA/kainate glutamate receptor antagonist CNQX (n=9). These findings are consistent with the involvement of TOPA quinone, an oxidative product of L-DOPA previously shown to excite non-dopaminergic neurons via AMPA/kainate receptors. Excitatory effects were greatly enhanced by glutamate transporter blocker TBOA (n=9), suggesting that L-DOPA also releases glutamate. The remaining excitation observed in the presence of both CNQX and NMDA receptor antagonist APV (evoked by 0.3 mM L-DOPA in 0.002 mM sulpiride), was not abolished by blockers of D1 (SCH-23390; n=12), alpha-1 adrenergic (prazosin; n=9) and mGluR1 (CPCCOEt; n=10) receptors. **Conclusion**: These results show that in the presence of D2 receptor blockade, L-DOPA excites nigral dopaminergic neurons mainly through activation of glutamate receptors, and suggest that the effect is due to both the direct action of TOPA quinone on AMPA/kainate receptors and to release of glutamate within the slice. The mechanism of the non-glutamatergic component of the response still needs to be elucidated.

POS-WED-122

DEVELOPMENT OF A PRIMARY MURINE NEURONAL-GLIAL CULTURE TO STUDY THE LIFE AND DEATH OF SEROTONERGIC NEURONES

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While the essential roles of brainstem serotonin (5-HT) neurones are well recognized, they have attracted renewed interest because of new evidence for key roles in autism, sudden infant death syndrome and regenerative neurobiology. Remarkably there is a dearth of knowledge about the life and death of 5-HT neurones presumably because they have been so difficult to study in primary culture. **Purpose**: (1) to develop methodology to allow the establishment of primary cultures containing 5-HT neurones, (2) to employ these cultures as suitable model to evaluate the patterns of injury and death of 5-HT neurones. **Method:** A coronal section of ventral brainstem, containing principally rostral groups of 5-HT-containing raphe nuclei, was dissected from the brains of E14-16 mice. Procedures for the digestion and isolation of cells were based upon those previously employed for mesencephalic cells (Mercer et al., Biochem Pharmacol 69: 339, 2005). Media changes were as previously described (Zagami CJ et al., Glia 57, 119, 2009). **Results:** Cultures possess mature neurones in the presence of astrocytes. Immunocytochemistry (n=6) for 5-HT, microtubule associated protein-2 (MAP2) and glial fibrillary acidic protein (GFAP) identified neurones which were MAP-2 and 5-HT positive, presumed 5-HT neurones, and mature astrocytes. The neuritic tree and primary axons of 5-HT neurones became increasingly complex over the 12 days in culture with very large neuritic trees. Ongoing work includes analyses of cell viability (n=3) and morphology, which indicate that 5-HT neurones are sensitive to injury by oxidative stress and autophagy. **Conclusion:** Our primary culture is suitable for dissection of the death modalities of 5-HT neurones.

POS-WED-123

MISMATCH NEGATIVITY (MMN) LIKE POTENTIALS IN THE WISTAR RAT: RELEVANCE TO NEUROPHYSIOLOGICAL ANIMAL MODELS OF SCHIZOPHRENIA

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Purpose: to determine whether MMN-like activity in the rat meets criteria for MMN, namely, that it reflects a memory-based comparison process and exhibits sensitivity to probability effects. MMN is a frontal negative deflection in ERPs generated to deviant sounds that violate regularities in background standard sounds. Reduced MMN is one of the most robust neurobiological findings in schizophrenia and meets many of the criteria for an endophenotype. As MMN is sensitive to the functional integrity of the glutamate NMDAR system, it is very relevant to models proposing that deficits in NMDAR function play a critical role in the pathophysiology of schizophrenia. Our overall goal is to establish a rodent model of schizophrenia and to assess the success of the model by the extent to which it produces a reduction in MMN in the adult animal and other NMDAR-related abnormalities. **Methods:** ERPs in alert Wistar rats (n=7, 6M) were recorded using 4 oddball conditions in which *regular background sounds* were occasionally (20%) interrupted by a deviant sound either a high/low frequency deviant, or a short/long deviant. Control conditions were employed for both frequency and duration oddballs in which *varying background sounds* intervened between deviants occurring at the same probability (20%) as the deviant oddball conditions. **Results:** MMN-like activity to high frequency deviants was found to meet the two crucial criteria, namely, a memory-based comparison process and sensitivity to local probabilities in terms of the number of standards preceding a deviant. The other deviant types did not. **Conclusion:** High frequency MMN shows promise as an endophenotype in animal models of schizophrenia.

POS-WED-124

$GABA_A$ RECEPTOR α1 AND α3 SUBUNIT EXPRESSION FOLLOWING NEONATAL HYPOXIA-ISCHAEMIA IN THE NEWBORN PIGLET

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Purpose: Treatment of seizures after neonatal hypoxia/ischaemia (HI) is one of the few therapeutic options available to doctors in the intensive care nursery. The principal function of the GABA system in mature brain is inhibition, however in the neonatal brain GABA provides much of the excitatory drive. Whilst anticonvulsants augment GABA's inhibitory actions in mature brain, administration of GABAergic drugs to neonates may exacerbate seizures and worsen HI brain injury. Differences in $GABA_A$ receptor expression will influence receptor pharmacology. We aimed to assess changes in protein expression of the $GABA_A$ receptor in the neonatal HI piglet model. **Methods**: Piglets (n=36) were subjected to a 30min HI insult and euthanased at 24 and 72h. HI animals were grouped based on presence or absence of seizure activity. Cortical brain tissue was collected and $GABA_A$ receptor α_1 and α_3 protein expression levels analysed by western blot. **Results**: $GABA_A$ α_1 and α_3 protein expression was altered temporally and regionally following HI. At 24h there were no differences in α_1 expression between HI, HI-seizure and control animals. At 72h α_1 expression was elevated in the parietal, temporal and occipital cortices of HI animals when compared with controls. At 24h α_3 expression was lower in the HI-seizure animals compared with HI animals without seizure, by 72h this reached significance ($p<0.05$). **Conclusions**: $GABA_A$ receptor α_3 expression was significantly altered following neonatal HI; presence of seizures further changed this expression. Further investigation into levels of protein expression of other α subunits is ongoing. There is a critical need to develop effective treatment strategies specific to the neonatal brain.

POS-WED-125

THE ROLE OF INTERFERON STIMULATED JAK-STAT PATHWAY IN NEURO-INFLAMMATION

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Introduction: Type 1 Interferons (IFNs) are a super-family of pleiotropic cytokines that induce pro-inflammatory gene transcription via the classical JAK/STAT pathway. Acute neuro-pathologies such as stroke and Traumatic Brain Injury (TBI) exhibit neuro-inflammation post-injury. Investigation of IFN-signalling in this neuro-inflammatory state has been limited. Furthermore, in brain tissue, the ligands and signalling events that elicit cell death through IFN-signalling remain unclear. **Purpose:** To investigate the involvement of interferon signalling in the progression of cell death following acute neural injury. **Methods:** Isolated C57-BL6 cortical neurons (wild-type and IFNAR1-/-) and human neuroblastoma (M17) cells were subjected to 4hr Oxygen Glucose Deprivation (OGD) or IFNα/IFNβ treatment (1000U/ml, 30mins). Post-OGD cells underwent reperfusion for 0, 0.5, 2 and 24hrs and were harvested for western blot analysis, RT-PCR and MTT assay. **Results:** Post OGD and 24hr reperfusion MTT assay shows increased viability of IFNAR1-/- neurons at 91.0±4.9% survival compared to wild-type with 49.78%±0.75 (n=6, P<0.05). M17s subjected to OGD and 2hr reperfusion showed prominent STAT-1, but not STAT-3, phosphorylation. To understand IFN-signalling after OGD M17 cells were exposed to IFNα/IFNβ to ascertain their STAT1/3 phosphorylation profile. Interestingly, IFNα stimulation induces STAT-1, but not STAT-3, phosphorylation at 10min, as opposed to IFNβ treatment phosphorylating both isoforms. To identify the time-course at which IFN contributes to the OGD response, RT-PCR was used to measure mRNA levels. IFNα mRNA was significantly increased by 12±4 fold at 2hrs reperfusion post-OGD (n=3, P<0.05), no increase with IFNβ. **Conclusion:** Collectively these results indicate signalling through the IFNAR1 subunit is deleterious in OGD. Furthermore, our findings suggest that IFNα driven phosphorylation of STAT-1 could be responsible for the resultant cell death signalling through IFNAR1 following OGD. Therapeutic agents targeting the IFNAR1 subunit may be beneficial in reducing the severity of a neuro-inflammatory event following stroke and TBI.

POS-WED-126

UNUSUAL PATTERNS OF FOS EXPRESSION IN THE SUBTHALAMIC REGION IN MPTP-TREATED MICE AFTER NEAR-INFRARED (NIR) LIGHT TREATMENT

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Purpose: We have shown previously that near-infrared (NIr) light treatment neuroprotects dopaminergic cells in the substantia nigra pars compacta (SNc) from degeneration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. The present study explores whether NIr light and/or MPTP treatment generates changes in Fos expression in the subthalamic region (subthalamic nucleus [Sub] and zona incerta [ZI]). **Methods**: BALB/c albino mice (n=40) were divided into 4 groups; (1) Saline (2) Saline-NIr (3) MPTP (100mg/kg) (4) MPTP-NIr. The injections were intraperitoneal and they were followed immediately by NIr light treatment (or not). Mice (n=40) were perfused transcardially with aldehyde fixative 6 days after their MPTP treatment. Brains were processed for tyrosine hydroxylase (TH) and Fos immunochemistry. Cell number estimated using the optical fractionator method. **Results**: The patterns of TH immunolabelling were similar to our previous study; briefly, there were ~50% fewer cells in the MPTP group compared to other groups. For Fos immunolabelling, in Sub and ZI, there were striking differences evident in Fos+ cell number in the different groups; in both nuclei, there were significantly more Fos+ cells in the MPTP compared to the Saline group. Further, there were also significantly more cells in the Saline-NIr and MPTP-NIr groups compared to the Saline group. In the case of Sub, the Fos+ cells occupied topographically distinct zones in the Saline-NIr and MPTP groups; in the Saline-NIr group, Fos+ cells were located ventromedially in the nucleus, while in the MPTP group, Fos+ cells were located dorsomedially. **Conclusion**: Our results indicated that both NIr light and MPTP treatment activated Fos expression in the Sub and ZI; moreover, that these two treatments appeared associated with different cell groups and perhaps distinct neural pathways.

POS-WED-127

THE EFFECT OF PHENCYCLIDINE TREATMENT ON NEUREGULIN1 AND ERBB4 RECEPTOR MRNA EXPRESSION IN THE ADULT RAT

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Phencyclidine (PCP), an NMDA receptor antagonist, is the best known drug that can mimic the range of symptoms of schizophrenia in humans and animals. Genetic linkage and post-mortem studies strongly suggest a role for altered neuregulin1 (Nrg1)/erbB4 signalling in schizophrenia pathology. This study investigated the relationship between the NMDA receptor and Nrg1 signalling pathways using the adult PCP animal model. **Purpose:** To determine if chronic adult PCP treatment affects Nrg1, erbB4 and NR1 mRNA gene expression, and NMDA receptor binding in rats. **Methods:** Male adult rats were treated with PCP (10mg/kg) or saline for 14 days. Rats were sacrificed (n=5/group) on drug withdrawal days 1, 3, 5, 7, 10 and 14. Nrg1, erbB4 and NR1 mRNA expression was analysed by in situ hybridisation, and NMDA receptor binding levels were measured by [3H]MK-801 receptor binding autoradiography. **Results:** PCP treatment did not affect Nrg1 or erbB4 mRNA expression. PCP slightly but significantly reduced NR1 mRNA expression across the majority of time points in the prefrontal cortex (7-24%, $p<0.05$), while in the hippocampus, NR1 mRNA expression was decreased at day 1 (~10% $p<0.01$), increased at day 5 (~13% $p<0.001$) and returned to control levels at day 14 after treatment cessation. PCP had no significant effect on NMDA receptor binding. **Conclusion:** While PCP treatment did not affect Nrg1 and erbB4 mRNA expression in the hippocampus, future research is required to determine its effect on expression and signalling of the Neuregulin1 pathway in other brain regions implicated in schizophrenia such as the prefrontal cortex.

POS-WED-128

UNDERSTANDING HOW SECRETED AMYLOID PRECURSOR PROTEIN-α (SAPPα) SIGNALS CHANGES IN GENE EXPRESSION IN NEURONS

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Purpose: Secreted amyloid precursor protein-α (sAPPα), a 612 amino acid brain protein, can be neurotrophic, neuroprotective and anti-apoptotic. The balance between sAPPα and the toxic amyloid-β peptide (both processed from APP) may be critical to the disease process in Alzheimers disease. RER, a peptide motif within sAPPα is reported to be capable of mimicking the functions of sAPPα. In this study we validate that sAPPα changes gene expression, and determine whether the RER motif can mimic these changes. **Methods:** The effects of sAPPα on three candidate genes, Insulin like growth factor-2 (IGF2), Insulin like growth factor binding protein-2 (IGFBP2) and Seladin-1, were modeled in cultured SHSY-5Y neuroblastoma (neuron-like) cells. Cells in triplicate were treated with either saline, recombinant sAPPα (2.5nM) or RER (10 nM) from 30min to 24h. The relative levels of the transcripts were determined in a SYBR Green quantitative PCR assay, where HPRT was the normalizing control gene. Data are an average of two separate experiments (n=6). **Results:** sAPPα treatment resulted in a transient increase in the expression of both IGF2 (5.5 fold, p<0.05), IGFBP2 (1.7 fold) and Seladin-1 (2.4 fold) after 30min but the increase in expression was not sustained after 60min. At 24h there was a secondary increase in expression of all three genes. RER peptide had similar but lower activity (IGF2 at 30min, 2.3 fold) than sAPPα. **Conclusion:** Both sAPPα and the RER motif as a peptide can induce gene expression changes in undifferentiated neuroblastoma cells, and the motif may play an important role in the protective properties of sAPPα in neurons.

POS-WED-129

MOLECULAR RESPONSE TO HYPOXIA IN ZEBRAFISH EMBRYOS

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Purpose: The aberrant splicing isoform, PS2V, is generated by exclusion of exon 5 from transcripts of the *PRESENILIN2 (PSEN2)* gene. It is expressed at higher levels in sporadic Alzheimer's disease brains. PS2V is induced by hypoxia in human neuroblastoma SK-N-SH cells. This induction can by blocked by antioxidants (Sato et al 1999 J. Neurochem. 72: 2498). Under hypoxic conditions HMGA1a, a non-histone chromatin binding protein, is induced and binds to a specific site in exon 5 of *PSEN2* transcripts to alter splicing and generate PS2V. Zebrafish embryos are tolerant of hypoxia and chemical hypoxia (e.g. induced using NaN3, CoCl2), making them a useful experimental model for studying cellular responses to hypoxia. The aim of this study was to examine: 1) whether induction of transcription of a zebrafish *HMGA1a* orthologous gene occurs under hypoxic conditions and, 2) whether this results in formation of a PS2V-like splice form of zebrafish psen2 transcripts. **Methods**: To examine these questions, zebrafish *hmga1* was identified and characterized and we established conditions for analysis of chemical hypoxia induced by NaN3 in zebrafish embryos. **Results**: Like its human counterpart, zebrafish hmga1 expression is induced under hypoxia and chemical hypoxia. However when zebrafish psen2 splicing was observed no PS2V-like isoform was detected. Instead, a hypoxia-sensitive splice variant was seen for zebrafish psen1.**Conculsion**: Future studies will focus on analyzing the effects of hypoxia and oxidative stress on other genes involved in sporadic Alzheimer's disease.

POS-WED-130

INCREASED PHOSPHORYLATED α-SYNUCLEIN RELATES TO ITS INSOLUBILITY IN MSA

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Purpose: Multiple system atrophy (MSA) is a progressive neurodegenerative movement disorder where there is elevated levels of monomeric and oligomeric α-synuclein protein in western blots of brain tissue, and phosphorylated insoluble α-synuclein inclusion pathologies in glia and neurons. The relationship between the levels of α-synuclein phosphorylation and changes in solubility in the brains of MSA cases has not previously been assessed. **Methods:** Frozen tissue from MSA (n=5) and age- and postmortem delay-matched control (n=5) white matter was obtained from the Australian Brain Bank Network following study approvals. Soluble and insoluble proteins were extracted and assessed by western blotting with specific phosphorylation-independent (Syn-1) and -dependent (P-S129) α-synuclein antibodies. Stepwise linear regression analyses were used to determine MSA-related protein changes. **Results:** Regression analysis revealed that, of the variables measured, increased white matter levels of soluble P-S129 (3.3x) and insoluble Syn-1 (6.6x) α-synuclein predicted MSA from controls (β coefficients=0.39 & 0.64 respectively, $p<0.001$). Soluble Syn-1 levels in the white matter significantly increased with increasing duration of MSA ($R=0.89$, $p=0.4$), doubling over time from control levels. **Conclusions:** Our data show for the first time that there is a large increase in soluble phosphorylated α-synuclein in MSA brain which is related to the levels of elevated insoluble α-synuclein, supporting the concept that phosphorylation of α-synuclein is important for insoluble aggregate formation in MSA. As the disease progresses, the levels of soluble phosphorylation-independent α-synuclein also increase, suggesting either enhanced α-synuclein production over time, or increasing deficits in clearance mechanisms in MSA.

POS-WED-131

CELL LOSS IN THE CEREBRAL CORTEX IN HUNTINGTON'S DISEASE

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Purpose: Huntington's disease is characterised by neuronal loss in the striatum and cerebral cortex, and a variable motor/mood symptom profile. This study measured neuronal loss in the superior parietal, primary visual, secondary visual, and middle temporal cortices of the Huntington's disease brain and compared the loss to the motor/mood symptom profile and neuropathological grade of each case. **Methods:** The pattern of cell loss was examined immunohistochemically using perfusion-fixed human cortical blocks from 14 Huntington's disease cases and 14 control cases stained for NeuN (total neurons) and quantified using stereological counting techniques. **Results:** Stereological cell counts demonstrated a significant total neuronal loss ($p<0.05$) in Huntington's disease in the superior parietal (36% loss), secondary visual (27% loss), and middle temporal cortices (27% loss) but not in the primary visual cortex, which showed no significant loss. The cell loss in the three affected regions increased with the grade of striatal neuropathology. Comparison of neuron number with motor/mood symptom profiles showed that in the **motor** Huntington's disease cases significant loss occurred only in the superior parietal cortex, while in the **mood** cases significant neuronal loss was observed in the superior parietal, secondary visual, and middle temporal cortices. **Conclusion:** These findings show that in Huntington's disease there is widespread cell loss across the parietal, occipital and temporal lobes of the cerebral cortex, with a variable pattern that reflects the variable symptom profiles exhibited by Huntington's disease patients.

POS-WED-132

SCREENING THE *THAP1* GENE FOR RARE SEQUENCE VARIANTS IN A PRIMARY DYSTONIA COHORT

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Purpose: Genes involved in familial dystonia syndromes are ideal candidates for investigating whether genetic variants influence the susceptibility to sporadic primary dystonia. Recently, novel sequence variants in the gene responsible for early-onset DYT6 dystonia (*THAP1*) were discovered in a large, mostly adult-onset primary dystonia case group. We decided to screen the THAP1 gene for sequence variants in an adult-onset primary dystonia case group from Queensland, Australia. **Methods:** Primary dystonia cases (n=233) were recruited from a movement disorder clinic in Brisbane, Australia. *THAP1* exons were screened using high resolution melt analysis (HRM). To validate the sensitivity of HRM as a screening tool, we designed assays for two *TOR1A* polymorphisms (rs2296793 and rs1801968), previously genotyped in this case group, and screened six individuals in triplicate. Sequencing of *THAP1* was performed in 20 random cases and served as reference samples for the *THAP1* assays. Melt curves that deviated from those of reference samples were subsequently rescreened and sequenced to confirm and identify the causative variant. **Results:** The *TOR1A* HRM assays were able to accurately detect the presence of both polymorphisms however it was unable to differentiate between heterozygotes and minor allele homozygotes for rs1801968. No unique melting curves were detected with the *THAP1* HRM assays. Sequencing confirmed the absence of sequence variants in all exons. **Conclusion:** Overall, we determined that HRM was sufficiently sensitive to use as a genetic screening tool. However, our findings suggest that sequence variants in the *THAP1* gene are not a common cause of primary dystonia in Queensland.

POS-WED-133

CEREBRAL HAEMORRHAGE UPREGULATES EXPRESSION OF MONOMERIC AND OLIGOMERIC FORMS OF Aβ IN HEALTHY BRAIN: IMPLICATIONS FOR PLAQUE FORMATION

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Purpose: Recent studies have developed the hypothesis that the 'senile' plaques found in aging human brain, and considered a characterisitic pathology of Alzheimer's dementia, form at sites of capillary haemorrhage. We have tested this hypothesis, testing whether haemorrhage induces plaque considered formation, and specifically the formation of oligomeric forms of Aβ precursors of the insoluble form found in plaques. **Methods:** Haemorrhagic lesions were made in the hippocampal and neocortex of young adult Sprague Dawley rats, using a stereotaxically directed needle. After 1-30d, the lesions were examined for haemorrhage, cell death, gliosis and the upregulation of Aβ using. Oligomeric Aβ was detected with an antibody (A11; Millipore) specific to oligomers. prefribrillar Aβ **Results:** Haem-rich deposits characteristic of haemorrhage formed along the needle track by 1d and persisted till the latest time point examined (30d). Upregulation of APP (amyloid precursor protein) and monomeric Aβ was apparent in the cytoplasm of neurones surrounding the track, but neuronal death was confined to the track. Extracellular deposits of oligomeric Aβ were prominent along the track at 1d. Oligomeric Aβ was also upregulated intracellularly, in neurones surrounding the track. The upregulation of APP, monomeric Aβ and oligomeric Aβ was transient, being prominent at 1-5d day post-lesion, and undetectable after 7 days. **Conclusion:** Haemorrhage in rat brain induces several features of human senile plaques, including haem depostion, gliosis, and the upregulation of mono- and oligomeric amyloid. The results support the idea proposed in earlier studies, that senile plaques in humans form at the site of haemorrhage from cerebral vessels.

POS-WED-134

CHARACTERISATION OF A TRANSGENIC OVINE MODEL OF HUNTINGTONS DISEASE

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A transgenic ovine model of Huntington's disease has been developed[1] to enable the examination of the earliest disease changes in a large mammal. *Ovis* were selected because their basal ganglia and cortex is similar to analogous regions of the human brain. Importantly, they live for more than a decade, allowing for the study of the chronic effects of a full-length HTT expressing transgene. The oldest animals are currently 4 years old, and are outwardly symptom free. **Purpose:** To examine transgene expression and repeat length stability in all transgenic progeny (G0-G3, n=150) by non-invasive methods, and to assess tissue expression patterns in the animals harvested (n=5) to date. **Methods:** Expression of the transgene has been assessed in transgenic progeny using QPCR and western analysis, and repeat length determined by sequencing. **Results:** The transgene mRNA and protein can reliably be detected in skin biopsy samples, and repeat length has remained relatively stable on transmission. The transgene transcript is expressed at levels close to that of the endogenous sheep HD transcript (1-2 fold), and transcript levels are higher in brain than in peripheral tissues. Robust transgene protein expression has been established throughout brain regions and peripheral tissues of the Kiwi line. **Conclusion:** Progeny of the Kiwi line stably express the transgene in brain and peripheral tissues. We are poised to investigate the early changes and molecular pathology of Huntington's disease in cohorts of animals now planned for longitudinal harvests. [1]Jacobsen et al 2010 Hum Mol Genet, 19:1873-82.

POS-WED-135

A PILOT TO DETERMINE THE PRACTICALITY OF USING MODIFIED GOLGI-COX STAINING TO ASSESS CORTICAL DENDRITIC MORPHOLOGY AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS

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PURPOSE: Whilst experimental stroke studies have showed much promise, a clinically effective acute treatment for stroke other than thrombolysis has yet to be translated into stroke patients. Most assessments of ischemic damage (and potential neuroprotection) have focussed on markers of the cell body with little attention given to damage of neuronal processes. Here we report a pilot using modified Golgi-Cox staining to examine the extent of damage to the dendritic arbour of neurons after stroke in rats. **METHODS:** Stroke was induced in normotensive WKY and hypertensive SHR rats by thread occlusion of the Middle Cerebral Artery (MCA)(n=19). 24 hours after MCAo the brain was collected and processed for modified Golgi-Cox staining. 150µm thick coronal sections were cut, and a section 0.2mm anterior of bregma used for staining. With the midline as a reference point, successive neurons spaced 500µm apart in layers III-V were assessed. **RESULTS:** Overall, WKY rats had greater dendritic branching than SHR (27±10 vs 18±8, mean±SD). Moving away from the midline, the ability to detect significant branching stopped sooner in the SHR than WKY consistent with prior reports of larger MCA infarcts in this strain. Within the limits of the sampling regime chosen, there were no differences in overall branching pattern between stroked and control hemispheres in either strain (WKY 31:27; SHR 18:18; contralateral:ipsilateral). **CONCLUSION:** Despite apparent differences at the level of simple visual examination, no differences were detected at microscopy. The confounding effects of significant numbers of cells with microglial morphology and a tendency to *pick* cells with something to measure probably accounts for these discrepancies. An unbiased sampling regime of cells in a volume of tissue is essential.

POS-WED-136

DOES DIFFERENTIAL EXPRESSION OF GIRK2 UNDERLIE CELL VULNERABILITY IN PARKINSON'S DISEASE?

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Background: Parkinson's disease (PD) results from the death of dopaminergic cells in the ventral rather than dorsal tier of the substantia nigra (SN)***[1]***. Based largely on rodent studies, we have suggested that this differential cell death may result from differences in protein expression between these tiers ***[1]*** with one such protein being the G-protein regulated inward-rectifier potassium channel 2 (GIRK2)***[2]***. **Purpose:** To determine whether GIRK2 protein expression differentiates neurons in the ventral and dorsal SN tier. **Methods:** Formalin-fixed midbrains from five brain donors aged 81.4 ± 2.3 without clinical or neuropathological abnormalities were obtained from the Sydney Brain Bank following study approvals. For comparison, C57BL6/J mice (n=5, 10 weeks, Animal Resource Centre) used. GIRK2 expression was investigated using single peroxidase and double fluorescence (with tyrosine hydroxylase, TH) immunohistochemistry in serial sections. **Results:** In humans, GIRK2 was observed mainly in the dendrites of dopaminergic neurons, with a greater density in the ventral (compared to the dorsal) SN tier. However, double labelling revealed that most of the GIRK2-positive dendrites overlapped with TH-immunoreactive dendrites in both SN tiers. In mice, GIRK2 was observed in TH-immunoreactive cell bodies. In both species, GIRK2 expression was not confined to the ventral tier but was found in almost all of the TH-immunoreactive SN neurons. **Conclusion:** These data show that GIRK2 expression does not selectively identify the more PD-vulnerable ventral SN tier in either humans or mice. Differences in anatomical delineation of SN likely underlie the discrepancy with previous studies. ***1.*** *Double et al. Prog. Neuro. Biol. 2010;92:316-29.* ***2.*** *Mendez et al. Brain. 2005;128:11498-510.*

POS-WED-137

RTMS CORRECTS BOTH FUNCTIONAL RESPONSE ANOMALIES AND TOPOGRAPHIC ERRORS IN AN ABNORMAL BRAIN CIRCUIT

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Purpose: Non-invasive and painless stimulation of brain tissue by administration of repetitive transcranial magnetic stimulation (rTMS) benefits a wide range of neurological and psychiatric disorders. However, previous investigations have been limited to short-term effects which contribute minimally to our understanding of long-lasting behavioural improvements in humans. Here we investigate the potential for rTMS to alter connectivity in a representative neural system, the mouse visual retinocollicular projection. Methods: We studied normal (wildtype; WT) and abnormal (ephrin-A2/A5$^{-/-}$ mice) projections. After 14 days (10min/day) of 6-9Hz stimulation to the superior colliculus, or sham treatment, the retinocollicular projection was assessed anatomically (anterograde tracing) and functionally (electrophysiological recording, visuomotor behaviour) in WT (n=40) and ephrin-A2/A5$^{-/-}$ (n=19) mice. In addition, levels of BDNF, nitric oxide and GABA in retina and superior colliculus were assessed by ELISA. Results: We found that rTMS altered anatomical and functional properties of the retinocollicular projection in ephrin-A2/A5$^{-/-}$ but not WT mice. rTMS treatment reduced the number of ectopic termination zones detected anatomically ($p<0.05$) but did not alter the number of functional ectopic points recorded physiologically ($p>0.05$). In addition, rTMS corrected the longer response latency in ephrin-A2/A5$^{-/-}$ mice and increased the size of the retinocollicular receptive fields ($p<0.05$). These changes were associated with significant changes in BDNF, nitric oxide and GABA in the retina and SC. Conclusions: We conclude that chronic treatment with rTMS can induce anatomical and functional improvements in an abnormal mature neural projection.

POS-WED-138

APOCYNIN TREATMENT DIFFERENTIALLY MODULATES SUPEROXIDE GENERATION IN INFLAMMATORY CELLS AND NEURONS AFTER ISCHEMIC STROKE WITH REPERFUSION

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NADPH oxidase is a major source of superoxide in rat brain after stroke and is differentially expressed in vessels, inflammatory cells and neurons. Apocynin is a specific NADPH oxidase Nox2 inhibitor and attenuates damage following stroke. **Purpose**: To investigate cell specific changes in superoxide generation following endothlin-1 (ET-1) induced stroke and treatment with apocynin. **Methods**: Apocynin (50 mg/kg, i.p. n=9) or vehicle (10% DMSO in saline, n=11) was given 1 h prior to stroke onset and again at 24 and 48 h after stroke. The middle cerebral artery was constricted by ET-1 in conscious rats (n=20). Neurological and histological outcome was assessed by neurological deficit score and MCID image analysis. *In situ* detection of superoxide using dihydroethidium (DHE) fluorescence was co-localised with markers for activated microglia/macrophages (OX-42) and neurons (NeuN). **Results**: Apocynin reduced the cortical infarct by ~60% ($P<0.01$; ANOVA) with no effect to striatal damage or functional deficits. DHE fluorescence revealed increased superoxide within the cortical infarct which was co-localized with both activated microglia/macrophages and neurons. Increased superoxide from activated microglia/macrophages after stroke was reduced in apocynin treated rats by ~ 58% ($P<0.001$; ANOVA), but further increased in surrounding NeuN positive cells by ~111% ($P<0.001$; ANOVA) compared to vehicles. Increased Nox2 immunoreactivity after stroke was associated with activated microglia and reduced in apocynin treated rats without change to cell numbers. **Conclusion**: Apocynin reduces inflammatory mediated damage after stroke through reduced Nox2 protein expression. Increased superoxide in neurons following stroke and treatment with apocynin is not Nox2 related, and may be associated with delayed spread of injury or compensatory changes in other NADPH oxidases.

POS-WED-139

SECRETED AMYLOID PRECURSOR PROTEIN-ALPHA REGULATES HIPPOCAMPAL GENE EXPRESSION

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Secreted amyloid precursor protein-alpha (sAPPα) is a neurotrophic and neuroprotective peptide generated by a processing pathway that precludes production of the Alzheimer's related neurotoxic peptide, amyloid-β. Inhibition of endogenous sAPPα synthesis *in vivo* results in a reduced retention of spatial memories and infusion of sAPPα *in vivo* enhances a cellular mechanism of memory, long-term potentiation (LTP). As both LTP and memory storage are dependent on new gene transcription, we hypothesised that the long-term effects of sAPPα may be mediated by altered gene expression. Purpose: To expand the known sAPPα-induced gene expression profiles in the hippocampus. Methods: Hippocampal organotypic slice cultures were treated with 1 nM sAPPα or vehicle control for 24 h (n=4) and the extracted RNA was hybridised to Affymetrix RAT Exon 1.0 ST microarrays. sAPPα-related gene expression profiles were produced using Limma analysis, $p<0.05$. The functional relationships of the genes within this profile were explored using Ingenuity Pathway Analysis. Results: The profiles comprised 29 up-regulated and 28 down-regulated annotated genes, suggesting that a limited set of genes are regulated 24 h after treatment. Canonical pathway analysis revealed that these genes were associated with axonal guidance, nervous system development, molecular transport, and cell-to-cellsignalling. Conclusion: Our data confirms that sAPPα alters the expression of individual genes and indicates potential mechanisms by which sAPPα contributes to enhancement of LTP as well as its neuroprotective and neurotrophic effects in the brain.

POS-WED-140

SEQUENTIAL INFUSIONS OF BMP4 AND NOGGIN DURING DEMYELINATION ALTER GLIAL CELL NUMBERS

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Purpose: A key pathological event in demyelinating diseases of the central nervous system is oligodendrocyte apoptosis, which leads to axons losing their myelin sheaths. Therefore, enhancement of oligodendrocyte regeneration by endogenous progenitor cells is a promising strategy for repair. Bone morphogenic proteins (BMPs) inhibit oligodendrogliogenesis *in vitro* and are increased in myelin lesions. We have recently found that BMP4 infusion increases oligodendrocyte progenitor cell proliferation during cuprizone-induced demyelination, while infusion of Noggin, its endogenous antagonist, promotes differentiation during recovery. Here, we report the effects of sequential infusions of BMP4 and Noggin during demyelination on numbers of oligodendrocytes and astrocytes following 1-week recovery. **Methods**: We used osmotic mini-pumps to infuse vehicle, BMP4 or Noggin in the following combinations: vehicle-vehicle, BMP4-vehicle, vehicle-Noggin and BMP4-Noggin. Lineage specific proteins were detected in tissue sections using immunohistochemistry. **Results**: Vehicle-Noggin infusion increased the density of Olig2-positive cells and Olig2-CC1 double positive cells in the corpus callosum compared to vehicle-vehicle, while the density of Olig2-positive cells was reduced in the BMP4-Noggin infused mice compared to vehicle-Noggin ($p<0.05$; n=3,5). BMP4-vehicle infusion increased the density of GFAP-positive cells compared to vehicle-vehicle, whereas BMP4-Noggin infusion decreased the density of GFAP-positive cells compared to BMP4-vehicle ($p<0.05$; n=3,4). **Conclusions**: These findings indicate that Noggin infusion increases the density of mature oligodendrocytes, while BMP4 infusion increases the density of astrocytes. Our results also suggest that BMP4 infusion can inhibit the generation of oligodendroglia by subsequent infusion of Noggin, while Noggin can decrease the BMP4-induced increase in astrocytes. We are currently examining the effects of the sequential infusions on remyelination.

POS-WED-141

REGIONAL DISTRIBUTION OF METALS IN THE HEALTHY HUMAN BRAIN

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Purpose: Metals, including copper, iron, and other transition metals, are involved in a range of neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease. Despite this, little information is available regarding the levels and distribution of metals in the human brain. **Methods:** In this study we quantified regional iron (Fe), copper, (Cu) zinc (Zn), manganese (Mn) and selenium (Se) concentrations in the brains of normal subjects (n=10) at different ages using inductively coupled plasma-mass spectrometry. **Results:** Metal levels varied in a regional manner and were not related to differences in post-mortem delay, brain pH or sex. The substantia nigra (SN) contained significantly higher levels of Cu, Fe and Zn, compared with other regions investigated. Compared with midlife, Cu levels in the aged SN were reduced and Se was increased. In the aged caudate, levels of Fe, Mn and Se were increased. **Conclusion:** These data suggest that the regional requirement for metals varies in the human brain and changes with normal aging.

POS-WED-142

MITOCHONDRIAL CONTRIBUTIONS TO NEURONAL INJURY OF CEREBELLAR GRANULE CELLS: EVIDENCE FOR RECRUITMENT OF AUTOPHAGY

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Involvement of dysfunctional mitochondria is recognized as a common theme amongst various neuropathologies. As key regulators of cell death and site of ATP generation, mitochondria via their respiratory chain and influences on programmed cell death (PCD e.g. apoptosis, autophagy) determine differential death outcomes of neurons. **Purpose:** to analyse mitochondrial roles in recruitment of PCD and to evaluate patterns of neuronal injury and death. **Methods:** Primary cultures of cerebellar granule cells (CGCs; Swiss mice) were exposed to insults targeting mitochondrial respiratory chain complexes I-IV (rotenone, 3-nitropropionic acid, antimycin A and KCN, respectively) and drugs that induce PCD: staurosporine (STS, apoptosis), rapamycin (autophagy) and H2O2 (oxidative stressor). Cell viability was determined by MTT assay and ATP content by bioluminescent detection of light in the presence of luciferin. Western immunoblotting for microtubule associated light chain 3-II (LC3-II) protein demonstrated the presence of autophagy, which was inhibited by 3-methyladenine (3-MA). Morphological analyses included Hoechst nuclear staining. **Results:** Insults produced concentration- and time-dependent reduction in cell viability (n≥3). Microscopy confirmed cell death with breakdown of neuronal networks and nuclear fragmentation. The decline in cellular ATP was time-dependent, occurring more rapidly with H2O2 than STS treated cells (n=2). LC3-II bands were detected in CGCs treated with rapamycin, STS and H2O2 (n=5). Inhibition of mitochondrial respiratory chain complexes, especially complex I and II, showed enhanced LC3-II bands (n=2), suggesting the involvement of autophagic mechanism. 3-MA decreased intensities LC3-II bands on immunoblots. **Conclusion:** Autophagic mechanisms are recruited to PCD by diverse cellular insults including those mediated via respiratory complexes.

POS-WED-143

EPHA4 ANTAGONISM PROMOTES FUNCTIONAL RECOVERY IN A RAT MODEL OF SPINAL CORD INJURY

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Purpose: We have previously shown that EphA4 knockout mice show remarkable functional recovery, axonal regeneration and decreased astrogliosis following a lateral hemisection spinal cord injury (SCI). These outcomes are duplicated in injured wildtype mice after treatment with the recombinant protein, EphA4-Fc. Here, we have assessed EphA4-Fc treatment in the rat following contusive SCI. **Methods:** Wistar rats received a moderate contusive SCI (150kDynes) at T10 and were treated for two weeks with vehicle (n=12) or 1mg (n=11), 5mg (n=12), or 20mg (n=11) of EphA4-Fc (per kg bodyweight, delivered every other day). Functional recovery was assessed by locomotor ability (BBB scale), balance beam assay, and weight gain. **Results:** The 1mg/kg and 5mg/kg groups showed improved locomotor ability at 1 and 3 days post-injury ($p<0.05$). At 120 days, all treated groups had higher average BBB scores, but this only reached significance when the treated groups were pooled ($p<0.05$). Rats treated with 5mg/kg or 20mg/kg EphA4-Fc showed improved ability on the balance beam ($p<0.05$). All treated animals regained pre-injury body weight more rapidly than untreated animals, but this was only significant for the 1mg/kg and 5mg/kg groups ($p<0.05$). **Conclusion:** EphA4-Fc treatment improved functional outcomes from contusive SCI in rats and thus continues to show promise as an effective therapeutic for SCI.

POS-WED-144

DYNAMIC RESPONSES OF OLIGODENDROCYTE PRECURSOR CELLS FOLLOWING OLIGODENDROCYTE APOPTOSIS

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Oligodendrocyte (OL) apoptosis has been proposed as a primary event in the etiology of multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS. To investigate this hypothesis, we have established a mouse model of inducible OL ablation. Transgenic mice expressing diphtheria toxin receptor regulated by the myelin basic protein promoter (MBP-DTR mice) exhibit specific OL ablation following DT challenge. We have already shown that OL ablation induces degenerative changes reflecting many aspects of MS. Partial restoration of functional deficits in MS is mediated via the generation of new myelinating OLs from oligodendrocyte precursor cells (OPCs) via poorly defined mechanisms. **Purpose:** To investigate regenerative responses initiated following OL apoptosis, we have examined the response of OPCs in the MBP-DTR model. **Methods:** MBP-DTR mice and wildtype littermates challenged with DT were perfused after 7, 14, 21, 28 or 35 days (n=4 per group). The density and morphology of OPCs (PDGFRα+/NG2+) in grey and white matter was assessed. **Results:** In the deep layers of the cortex, we observed a significant increase in OPC density and process outgrowth in MBP-DTR mice at 7 days compared with 28 days ($P<0.05$). In the lateral corpus callosum of MBP-DTR mice, there was a significant increase in somal area of OPCs at 7 days compared with 14 days ($P<0.05$), and in OPC process outgrowth at 7 days compared with 21 and 28 days post-DT ($P<0.05$). Conclusion: Our data indicate that OPCs enter the cell cycle and undergo morphological changes rapidly following OL apoptosis. Elucidating the molecular mechanisms orchestrating the response of OPCs to OL apoptosis could help identify new therapeutic targets to potentiate CNS repair.

POS-WED-145

IDENTIFICATION AND EXPRESSION ANALYSIS OF A ZEBRAFISH ORTHOLOGUE OF KLOTHO

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Purpose: Klotho is a multi-functional protein whose functions are involved in ageing. Klotho deficiency in mice causes accelerated ageing while its overexpression extends longevity. In humans, polymorphisms in *KLOTHO* are associated with longevity. The zebrafish is a useful model organism for studies of development and human disease, and we have begun investigation of Klotho using this organism. **Methods**: The zebrafish genome was searched for an orthologue of Klotho using mouse and human Klotho protein sequences as probes. To confirm a putative candidate gene as a zebrafish orthologue of Klotho, phylogenetic and synteny conservation analysis were performed. To validate the predicted transcript *in vitro*, the entire open reading frame of zebrafish klotho was amplified and sequenced. To determine the spatiotemporal expression patterns of *klotho*, RT-PCR in embryos and various adult tissues was conducted. **Results**: The candidate gene for a zebrafish orthologue of Klotho was identified on chromosome 10. Phylogenetic and synteny analysis confirmed the candidate gene as the zebrafish orthologue. Sequencing data predicts that the zebrafish klotho encodes a 937 amino acid residue protein. Alignment of zebrafish Klotho with human and mouse Klotho sequences showed considerable structural similarity. RT-PCR revealed that *klotho* is expressed from an early stage of development and in several tissues in adult zebrafish. **Conclusions**: This is the first evidence for identification of a zebrafish orthologue of Klotho. The orthologue has a similar structure and expression pattern to the human and mouse genes except that it is expressed during embryogenesis. We can exploit the many valuable characteristics of the zebrafish model to investigate the anti-ageing functions of the Klotho protein and its role in neurodegeneration. We are currently attempting to mutate *klotho* using zinc finger nuclease technology.

POS-WED-146

INTERACTION OF BENZODIAZEPINE AND FLUOXETINE ON NEUROGENESIS IN THE SOCIALLY ISOLATED MODEL OF DEPRESSION

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Patients with depression are often co-administered antidepressant agents with a benzodiazepine to reduce associated anxiety. Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine have been shown to reduce GABA inhibition and augment BDNF levels resulting in increased hippocampal neurogenesis and neural plasticity. However co-treatment of SSRIs with a benzodiazepine may oppose the effect of SSRIs on GABAergic inhibition and BDNF expression, thereby preventing improvement in hippocampal neurogenesis. **Purpose:** Using a rodent model of chronic stress, this study compared the effect of combined SSRI/benzodiazepine treatment on anxiety/depressive-like behaviour and hippocampal neurogenesis to treatment of SSRI or benzodiazepine alone. **Methods:** Rats (n=10/group) were subjected to 6 weeks of socially isolation (SI) before being treated either with benzodiazepine, fluoxetine, or combined benzodiazepine/fluoxetine for 3 weeks. Anxiety/depressive-like behaviour was assessed using the novelty-suppressed feeding (NSF) test and forced-swim (FS) test. Animals were injected with thymidine analogues to assess progenitor cell proliferation and survival. **Results:** More fluoxetine-treated animals improved in the NSF test, while more benzodiazepine animals improved in the FS test. In contrast, combined fluoxetine/benzodiazepine treatment resulted in the greatest number of animals exhibiting no change or worsening of behavioural profile. While fluoxetine significantly increased progenitor cell survival compared to untreated animals ($p<0.05$), this effect was not seen with benzodiazepine alone or combined benzodiazepine/fluoxetine treatment. **Conclusion:** Co-treatment of chronically stressed animals with fluoxetine and benzodiazepine prevents improvement in both anxiety/depressive-like behaviours and hippocampal neurogenesis when compared to treatment with fluoxetine alone. This suggests use of benzodiazepines may impede the molecular effects of SSRIs and reduce clinical efficacy.

POS-WED-147

EXPRESSION OF KYNURENINE PATHWAY GENES IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MICE TREATED WITH KYNURENINE PATHWAY ANALOGUES

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Multiple Sclerosis (MS) is a demyelinating inflammatory disease condition of central nervous system. Experimental Autoimmune Encephalomyelitis (EAE) is an animal model of MS, resemble various forms and stages of MS very closely in a large number of ways. In this study, C57BL/6 mice were induced with EAE (n=6/group/time point) as form of chronic disease condition by using myelin oligodendrocyte glycoprotein (MOG). MOG induced EAE mice were treated with kynurenine analogues such as 1-methyl tryptophan, tranilast and Ro 61-8048 for 3 and 7 days. After 3 and 7 days treatment period, the mice brain were harvested and analysed for kynurenine pathway (KP) genes such as indoleamine 2,3-dioxygenase (IDO1), 3-hydroxyanthranilate 3,4-dioxygenase (3HAO), kynurenine aminotransferase I (KAT1), kynurenine aminotransferase II (KAT2), kynureninase (KYNU), kynurenine 3-monooxygenase (KMO) and tryptophan 2,3-dioxygenase (TDO). RPL13 (60S ribosomal protein L13) were used as housekeeping gene. The results showed that the expression of KP genes were attenuated in treatment groups compared to control. Overall, based on the gene expression data and clinical disease condition, kynurenine analogues treated groups have had effect on impact of EAE disease severity compared to untreated control which clearly depicted that there is an interaction between kynurenine pathway and MS.

POS-WED-148

CHRONIC ACTH STIMULATION MODULATES TRICYCLIC ANTIDEPRESSANT EFFICACY AND PREFRONTAL CORTEX MONOAMINE LEVELS IN THE FORCED SWIM TEST

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Major Depressive Disorder is a disabling condition characterised by lowered mood, anhedonia, irritability and abnormalities in sleep and appetite. While knowledge about the neurobiology of depression has greatly improved in recent years, the pathophysiological mechanisms mediating the treatment-resistant state remain unclear. Treatment-resistant depression is associated with hyperactivity of the hypothalamic-pituitary-adrenal axis and chronic adrenocorticotrophin (ACTH) treatment blocks tricyclic antidepressant efficacy in the forced-swim test (FST). Here were confirmed this behavioural effect and measured monoamine levels in the left and right prefrontal cortex (PFC) 1 hour following exposure to the FST. Chronic (14 day) treatment with ACTH (100µg/day) effectively blocked imipramine (10mg/kg) mediated reductions in immobility in the FST. Animals were euthanased 1 hour after exposure to the FST stress. Post mortem tissue levels of serotonin and noradrenalin were demonstrated to be significantly elevated in ACTH-treated animals relative to controls. A non-significant trend towards reduced monoamine levels in the left PFC was observed for all animals. Further work is needed to establish the efficacy of other antidepressant strategies in this animal model, and elucidate the mechanisms through which chronic ACTH mediates antidepressant efficacy in the FST.

POS-WED-149

NOT JUST REUPTAKE. SELECTIVE SEROTININ REUPTAKE INHIBITORS ATTENUATE LPS INDUCED MICROGLIAL PRO-INFLAMMATORY CYTOKINE PRODUCTION

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Purpose Increasing evidence from preclinical investigations suggest that microglial activation accompanied with subsequent elevations in the synthesis of proinflammatory mediators may play a pivotal role in the pathogenesis of depression. In support of this recent evidence, both peripheral and central administration of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) induce transient depressive episodes in humans, and sickness behaviour in rats (Danzter, 2004). The current study aims to evaluate potential anti-inflammatory properties of one of the most commonly used pharmaceutical compounds used to treat depression. Specifically, the study will assess the capacity of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), to decrease lipopolysaccharide (LPS) activated microglial cells: (1) production of the pro-inflammatory cytokine TNF-α; (2) phagocytic activity; and (3) alterations in their chemotactic profile. **Method** Murine BV-2 microglial cells were co-cultured with LPS (10ng/mL) and FLX (0-100μM) for a 24 hr period. Cell free supernatant was collected and assayed using ELISA to determine TNF-α production. Microglial migration was measured in response to monocyte chemoattractant protein 1 in a chemotaxic chamber. Phagocytic potential was determined by analyzing the uptake of co-cultured fluorescent microbeads. **Results** The results indicated a significant increase in TNF-α production in cells exposed to LPS ($p<.01$). MTT analysis indicated that this concentration of LPS did not impact cellular viability ($p<.05$). Preliminary results indicate the SSRI fluoxetine significantly decreases TNF-α synthesis ($p<.01$) in a concentration-dependant manner. **Conclusion** These results demonstrate the efficaciousness of BV-2 microglial cells for assessing functional properties of microglia. We are now in the process of establishing whether this inhibitory relationship generalizes to other microglial activities including migration and phagocytosis. The information derived from the current study will contribute to our understanding of the mechanisms underlying anti-depressant treatment, with the aim to enhance currently available treatment options.

POS-WED-150

CONNEXIN43 MIMETIC PEPTIDE DELIVERY: A NOVEL THERAPEUTIC STRATEGY FOR ISCHEMIC STROKE

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Gap junction hemichannels, in particular those formed by the gap junction protein Connexin43 (Cx43), contribute to the propagation of neuroinflammation in a wide range of neurological disorders including ischemic stroke. We propose that regulation of gap junction hemichannel function to modulate neuroinflammation provides a novel strategy for the treatment of ischemic stroke. We can block Cx43 gap junction hemichannel function using a mimetic peptide. **Purpose:** To investigate the effect of Cx43 mimetic peptide delivery on ischemic brain injury and the survival of newly generated neurons. **Methods:** Cx43 mimetic peptide (5, 10, 20 and 50 μmol/kg) or vehicle solution was delivered into the lesioned striatum of mice immediately following transient middle cerebral artery occlusion (tMCAo). The temporal expression of Cx43 mRNA was investigated in untreated animals following tMCAo using quantitative PCR. **Results:** Cx43 mimetic peptide (10 μmol/kg, n=5) significantly reduced brain atrophy compared to vehicle ($P\leq0.01$, n=5). This suggests that transient blocking of Cx43 hemichannel function immediately following tMCAo can reduce tissue loss. However, the proportion of newly generated neurons in the lesioned striatum was not enhanced. We observed an increase in Cx43 mRNA expression from 24 to 72 hours post-tMCAo ($P\leq0.05$, n=3/group), suggesting a new treatment time for targeting regenerative processes. **Conclusion:** Delivery of Cx43 mimetic peptide may be necessary at different time points following focal ischemia to reduce atrophy or to support regeneration. These initial results support further investigation into Cx43 mimetic peptide as a therapeutic strategy for ischemic stroke.

POS-WED-151

DELAYED DELIVERY OF A MIMETIC PEPTIDE AGAINST CONNEXIN43 IS PROTECTIVE IN A RAT MODEL OF SPINAL CORD CONTUSION INJURY

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Purpose: Connexin43 is a gap junction protein up-regulated after spinal cord injury (SCI), leading to lesion spread. We have previously shown that delivery of connexin43 mimetic peptide immediately after injury to an *in vivo* rodent spinal cord contusion model demonstrated a transient improvement in locomotor scores, reduced lesion size, reduced astrocytosis and increased neuronal survival. To be clinically relevant, however, we need to determine the window of opportunity for delivery after injury. **Aim:** To test whether delayed delivery of mimetic peptide would be protective. **Methods:** Rats (n=20) were subjected to a 10g, 12.5 mm weight drop injury at the vertebral level T10. One hour following injury, vehicle or connexin43 mimetic peptide (20 μmole/kg) was delivered to the lesion site over one hour followed by 20 μmole/kg at a rate of 8μl/hr over 24 hours. Animals were killed 6 weeks post injury and tissue collected for immunohistochemical analysis. **Results:** A significant ($p < 0.05$) reduction in the size of lesion area was seen. Down regulation of Cx43 ($p < 0.001$), a reduction in astrocytosis (GFAP) ($p < 0.001$) and the number of activated microglia (Iba1/ED1) adjacent to the lesion ($p < 0.001$) was also observed in peptide treated samples compared to vehicle controls. A marked increase in the length of white matter fibre tracts, as assessed by staining with the neurofilament marker SMI32, was seen in peptide treated animals compared to controls. **Conclusions:** These results indicate the clinical potential of connexin43 modulation using mimetic peptides to improve outcomes following SCI.

POS-WED-152

TREATING SYSTEMIC HYPERTENSION AND STROKE INJURY BY NADPH OXIDASE ANTAGONIS

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It is widely recognised that oxidative stress is the leading pathomechanism of cardiovascular diseases (CVD). However, clinical trials with antioxidant supplements have not yielded positive results in the treatment of CVD and risk factors such as systemic hypertension and stroke injury. Here, we identify NADPH oxidases (NOX) as the relevant source of reactive oxygen species (ROS) in hypertension and ischemia/reperfusion (I/R) damage. Therefore, inhibiting the source of oxidative stress offers a novel approach in the prevention and management of stroke injury. **Methods and Results**: Aged spontaneously hypertensive rats (SHR) showed endothelial dysfunction and increased vascular ROS levels compared to aged-matched WKY rats. Pharmacological inhibition of NADPH oxidases with VAS2870 normalised aortic ROS levels and restored endothelial function in SHR. There was a significant upregulation of vascular NOX1 and NOX2 isoforms in the SHR aortas. Interestingly, NOX4 levels were unchanged, while NOX4 knockout (KO) mice displayed normal blood pressures. In contrast, NOX1 displayed ectopic expression in the endothelium of aged SHR. However, a major pathophysiological role for NOX4 became evident in I/R induced tissue injury: NOX4 was upregulated after I/R in both human and mouse brains. After acute and chronic ischemia NOX4 KO mice were protected from signs of brain injury including blood-brain barrier damage neuronal apoptosis. Importantly, pharmacological intervention to inhibit NOX within a clinically relevant time frame, i.e. post-stroke, protected mice from stroke-induced I/R injury. **Conclusions**: NOX1 and NOX4 offer potential targets for the treatment and prevention of oxidative stress-related hypertension and I/R injury, respectively.

POS-WED-153

CHRONIC STRESS MODULATION OF MICROGLIAL-NEURONAL INTERACTIONS: IMPLICATIONS FOR DEPRESSION

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Purpose: Several recent reports have identified that psychological stress can both structurally and functionally alter microglia, cells that are pivotal to the production and maintenance of a neuroinflammatory state in the brain. The ability of stress to modulate microglial activity is of interest for two main reasons (a) stress is major risk factor in the emergence of depression and (b) depression appears to be characterised by enhanced levels of neuroinflammation. These two facts have led to the hypothesis that psychological stress may elicit changes in mood state and cognitive function by driving microglial mediated neuroinflammatory events. **Methods;** In investigating this hypothesis our research group, using a variety of behavioural approaches, has previously found that chronic stress sufficient to induce an increase an anhedonic status and a decline in cognitive performance co-occurred with an increase in microglial activation within mood regulatory forebrain nuclei (notably the medial prefrontal cortex and amygdala). **Results:** We have now subsequently, established that targeting stress induced microglial activation with anti-inflammatory agents improves stress induced cognitive decline. Moreover, using a variety of immunohistochemical techniques we have identified at a cellular level that microglial activity is intimately linked with neuronal activity. Interestingly, we have also observed that the stress induced changes in microglial activity are not clearly associated with signs of neurodegeneration. Indicating that the stress induced increase in microglial activation is occurring via a non classical mechanism. Currently, our group is now functionally characterizing, using a variety of ex-vivo techniques, the inflammatory status of microglia within the mood regulatory nuclei where we have observed differences following exposure chronic stress. **Conclusion:** Collectively, these findings may prove to be relevant in furthering our understanding of the neurobiology of depression.

POS-WED-154

IMPLANTED RAT NEUROEPITHELIAL CELLS PROMOTE SCARRING IN THE INJURED RAT SPINAL CORD

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Spinal cord injury results in the loss of both sensory and motor function within the central nervous system (CNS). The mammalian CNS does not have the ability to regenerate axons and therefore spinal cord damage is both devastating and permanent. Recent research suggests that early embryonic spinal cords cells, known as Neuroepithelial cells (NE), may have the potential to stimulate injured axons and encourage growth after injury. **Aim:** to examine the growth promoting potential of NE cells at embryonic day (E) 11.5 after adult rat spinal cord injury. **Method:** Rat NE cells were dissected from E11.5 embryo's, dissociated and implanted ($1x10^5$ cells per rat) via Hamilton's syringe 1 mm proximal to an adult rat spinal hemi-section injury (n=8). 6 weeks after injury rats received cortical injections of the axonal tracer Biotin Dextran, to label corticospinal tract axons. 2 weeks after tracing, rats were sacrificed and perfused fixed with 4% Paraformaldehyde. Immunohistochemistry was performed for Avidin Peroxidase and counterstained with cresyl violet to visualise sprouting axons within the implant site. **Results:** Results show a significant difference in spinal cord volume between treatment and control groups. There is also a significant difference in volume between control and NE implant sham groups. These results suggest that NE cells have an uncontrolled growth potential and without sufficient guidance promote significant scarring within the spinal cord in both injured and non injured animals. Paradoxically, results also suggest NE cells have the ability to form neural pockets, resulting in a positive effect on axonal outgrowth. **Conclusion:** Cellular phenotype is critical when implanting embryonic tissues. A neural phenotype strongly promotes growth while a scarring phenotype is inhibitory.

POS-WED-155

UTILISING ZEBRAFISH TO INVESTIGATE PRESENILIN ACTIVITY FOR ALZHEIMER'S DISEASE RESEARCH

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Aberrant proteolytic processing of AMYLOID PRECURSOR PROTEIN (APP) by γ-secretase complexes results in an imbalance between production and clearance of the Aβ proteolytic product. This appears to promote neuronal dysfunction and death. Presenilin proteins form the catalytic cores of γ-secretase complexes. Genetic studies have discovered mutations in *APP* and *PRESENILIN 1* and *2 (PSEN1, 2)* that contribute to familial autosomal dominant AD. The zebrafish, *Danio rerio*, is a versatile vertebrate model for investigating the molecular bases of AD pathology. It possesses genes orthologous to human *PSEN1* and *2*, and *APP*. We have demonstrated previously that disruption of *PSEN1* transcript splicing in zebrafish can have potent dominant negative effects on the function of *PSEN1* and the related gene *PSEN2*. We hypothesise that the aberrant splicing of *PSEN1* and/or *PSEN2* transcripts in aging or stressed neural cells may contribute to AD by producing truncated proteins that act in a dominant negative manner on Aβ production by invading γ-secretase complexes. We have investigated this by manipulating splicing using morpholino antisense oligonucleotides and by injection of synthetic mRNAs encoding *PSEN1* and *PSEN2* truncations. We have shown that particular truncations differentially affect *PSEN*-related activities, particularly APP and Notch processing. Currently there is no *in vivo* assay appropriate for investigating γ-secretase activity in zebrafish. However utilising a modified APP substrate, where changes in the cleavage of the substrate can be quantified, allowed *in vivo* analysis of the effect of truncated *PSEN1* on γ-secretase activity. The induced/injected truncations have been shown to incorporate into higher molecular weight complexes via western blotting, immunoprecipitation and proteomic analyses.

POS-WED-156

MULTIPLE ACTIVATION STATES IN PRIMARY CULTURES OF RAT MICROGLIA DEFINED USING CALCIUM IMAGING

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Purpose: Microglia are macrophage-like immune cells of the CNS and in vivo require activation by neuronal damage or infection before releasing inflammatory mediators. Their activation is inhibited by minocycline in vivo. In culture, activation with bacterial lipopolysaccharide (LPS) is associated with a sustained increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$), but ATP is also known to activate microglia. We therefore investigated the effects of ATP and minocycline on intracellular calcium in cultured microglia. **Methods:** Microglia (98% pure) were isolated from mixed glial cultures of neonatal rat brain by shaking. Intracellular calcium was then measured using fura-2-AM and ratiometric Ca^{2+} imaging. **Results:** Cells from different cultures showed different initial baseline $[Ca^{2+}]_i$ suggesting some were partially activated. Brief applications of ATP (50uM, 30s) produced transient increases in $[Ca^{2+}]_i$, the amplitudes of which were negatively correlated with initial $[Ca^{2+}]_i$ (n = 205). Minocycline (30-100uM) reduced both the initial peak response to ATP and the sustained increase in $[Ca^{2+}]_i$ seen after a 1hr application. In cells with a high initial $[Ca^{2+}]_i$ however, a brief application of ATP was enough to cause a significant elevation in $[Ca^{2+}]_i$ 1 hour later. When applied 5 minutes after a brief ATP application, minocycline could no longer inhibit either the peak response to ATP or the change in baseline over 1 hour. **Conclusion:** Cultured microglia show multiple activation states. When partially activated, microglia are highly sensitive to further activation. In this state minocycline can only prevent the effects of ATP if applied beforehand. Hence our results support the idea that minocycline can help prevent but not reverse pathological changes due to microglial activation.

POS-WED-157

DELAYED OLFACTORY ENSHEATHING CELL TRANSPLANTS MITIGATE DEAFFERENTATION PAIN FOLLOWING DORSAL ROOT INJURY

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Injury to cervical dorsal roots mimics the deafferentation component of brachial plexus injury in humans, with intractable neuropathic pain in the deafferented limb being a common consequence. Such lesions are generally not amenable to surgical repair. The use of olfactory ensheathing cells (OECs) for dorsal root repair, via acute transplantation, has been successful in several studies. From a clinical point of view, delayed transplantation of OECs would provide a more realistic time-frame for repair. In this study we investigated the effect of delayed OEC transplantation on functional recovery of skilled forepaw movements and amelioration of neuropathic pain, using a C7 and C8 dorsal root injury rat model previously established in our lab. We found that OEC transplantation to the dorsal horn 1 week after root injury effectively attenuated neuropathic disturbances associated with dorsal root injury, including spontaneous pain behavior, tactile allodynia and thermal hyperalgesia. The sensory controls of complex, goal-oriented skilled reaching and ladder walking, however, were not improved by delayed OEC transplantation. We did not detect any significant influence of transplanted OECs on injury-induced central reorganisation and afferent sprouting. The anti-nociceptive effect mediated by OEC transplants may therefore be explained by alternative mechanisms such as modification of inflammation and astrogliosis. The significant effect of OEC transplants in mitigating neuropathic pain may be clinically useful in intractable pain syndromes arising from deafferentation.

POS-WED-158

ENHANCING AXON REGENERATION AFTER SPINAL CORD INJURY WITH THE USE OF CONNEXIN43 ANTISENSE OLIGODEOXYNUCLEOTIDES

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Purpose:Spinal cord injury is comprised of the initial injury and the secondary effects including injury spread, swelling, inflammation, and scarring. Connexin43 gap junctions and hemichannels contribute significantly by remaining open in dying cells and spread toxic substances to neighbouring cells and the extracellular environment. Peripheral nerve grafts have been found to successfully promote axon outgrowth from spinal cords, but regeneration is limited due to scarring at the site of injury. In our study, the effect of Cx43 down-regulation on preventing the onset of secondary injury spread and enhancing peripheral nerve graft repair was investigated. Methods: An *ex vivo* spinal cord segment culture model was established utilizing rat spinal cords cultured in media for five days. The segments were treated with Cx43 antisense oligodeoxynucleotides (Cx43 AsODN) resulting in viable, organotypic, air-liquid interface cultures that can be used for repair studies. After incubation, spinal cords were cryosectioned and stained immunohistochemically. Fresh peripheral nerves were grafted into the spinal cord segments (n=6) with Cx43AsODN applied concomitantly, followed by five days of culture and subsequent processing. Results: Results have shown that spinal cord segments cultured in the presence of Cx43 AsODN have significantly less swelling than control spinal cords, indicating the efficacy of this treatment in reducing initial cellular and tissue swelling. Improved neuronal survival and neural sprouting from the injured spinal cord into the grafted peripheral nerve, along with a reduction in glial cell response, was seen in this Cx43 AsODN treated organotypic culture model.*In vivo* T10 complete transection rat studies are underway and preliminary results indicate that Cx43 AsODN treatment following pre-conditioned sciatic nerve grafting reduced lesion cavity formation and enhanced axon regeneration after 6 weeks. Conclusion: Cx43 AsODN offers a promising treatment for attenuation of secondary injury processes when used in conjucntion with other spinal cord injury repair treatments.

POS-WED-159

COMPARING LONGITUDINAL AND TRANSVERSE MODES OF EXTRCELLULAR ELECTRICAL STIMULATION OF NEURAL FIBRES

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Purpose: Neuroprosthetic devices, such as cochlear and retinal implants, work by directly stimulating neurons with extracellular electrodes. This is commonly modelled using the cable equation with an applied extracellular field. A potential problem with this approach is that the cable equation implicitly assumes the applied extracellular field is rotationally symmetric about the cylinder's axis, which is only true in atypical situations. In most cases the transverse component of the field is much larger. We compare the predicted magnitudes of depolarisation for transverse versus longitudinal stimulation. **Methods**: Laplace's equation is solved in three dimensions in the intra- and extra-cellular space surrounding a cylindrical membrane cable. The solution follows an expansion in cylindrical harmonics. **Results**: Under reasonable assumptions, the zero-th order solution recovers the cable equation, which describes the longitudinal mode of stimulation resulting from the rotationally symmetric component of the extracellular field. The first order solution is a simple ordinary differential equation in time driven by the (spatially varying) transverse component of the extracellular field. It describes the transverse mode of stimulation. Assuming a point source electrode, simple analytical approximations are given for the peak depolarization of the longitudinal and transverse modes of the membrane potential. **Conclusion**: They show that these two modes are comparable in magnitude provided that electrode is no closer than 100 microns to the fibre and the stimulation pulse is no longer than a few hundred microseconds. This substantiates the need for our extended formalism.

POS-WED-160

SELECTIVE TARGETING TO ASTROCYTES BY ADENO-ASSOCIATED VIRAL VECTOR SEROTYPE 9

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Purpose: Recombinant adeno-associated viral (AAV) vectors have become the gene delivery vehicles of choice for human gene therapy to the central nervous system (CNS). The appreciation that astrocytes play a vital role in the function of the nervous system, and astrocytic dysfunction contributes to the progression of neurodegenerative diseases has important implication for the design of AAV-based gene therapy approaches. Given that extensive neuronal loss and reactive astrogliosis are defining features of neurodegenerative diseases, astrocytes may be a better cellular target for CNS gene therapy. We have previously identified three non-human primate derived AAV serotypes rh43, 8 and 9 that display propensity for astrocytic transduction. The aim of this study was to extend our characterization of in vivo astrocytic transgene expression mediated by these vectors. **Method:** AAVrh43, AAV8 and AAV9 vectors expressing a luciferase or green fluorescent protein (GFP) were injected into the hippocampus and striatum of adult Sprague Dawley rats (n > 5 per injection site). AAVrh43-mediated expression of neuropeptide Y (NPY), a potential therapeutic transgene for gene therapy of temporal lobe epilepsy was also investigated. Three weeks following vector infusion, brains were taken for analysis. **Results:** AAVrh43 mediated expression of GFP exclusively in astrocytes as found previously, but this serotype failed to mediate detectable levels of luciferase or NPY expression in the rat hippocampus. In comparison to AAV8, widespread astrocytic transduction in the hippocampus and striatum was achieved with both AAV9 vectors expressing luciferase or GFP. **Conclusions:** These results suggest that AAV9 vectors could be an optimal vector system to enable genetic manipulation of astrocyte function as a therapeutic approach for neurodegenerative diseases.

POS-WED-161

DIFFERENTIAL LENTIVIRUS-MEDIATED GENE TRANSDUCTION EFFICIENCY BETWEEN NEURONAL SUBTYPES IN THE JUVENILE RAT STRIATUM

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Purpose: The development of viral vectors to efficiently deliver genes to neurons *in vivo*, and the creation of optically controlled ion channels for the precise manipulation of excitability in specific cell types make it possible to manipulate gene function or cellular activity in specific brain regions. Here we test the feasibility of establishing a gene knockdown strategy that targets cholinergic neurons of the rat striatum representing a small subpopulation (< 1 %) of striatal neurons. **Methods:** Lentiviral vector was stereotaxically injected into the striatum of 10-day-old rat pups, brains perfused with fixative two weeks later, and the degree of fluorescent reporter gene expression in neurons in general, and specifically in cholinergic interneurons estimated using stereological techniques, and double immunolabelling with NeuN and ChAT, respectively. **Results:** The spread of reporter gene expression was found to be 3.3 and 5.9 mm^3, using 2 and 4 µl lentiviral injections, respectively (n = 2 for each condition). This equates to 22 and 39 % of the total striatal volume of 15 mm^3. The density of transduced cells in both groups was similar at 42,706 ± 6133 cells/mm^3, and on average 34% of neurons within the affected volume were found to express the GFP transgene. Of the transduced cells, 78 % were neurons but GFP transgene expression was found in only 1 % of the cholinergic interneurons in the affected region. **Conclusion:** These results suggest that lentivirus mediated gene transfer provides an efficient means to target principal neuronal classes but some neuronal cell types appear more receptive to viral transduction than others.

POS-WED-162

MAKING SENSE OF IN VITRO CULTURES OF THE ADULT HUMAN BRAIN

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PURPOSE: The recent advances in cell culture techniques have allowed adult human brain tissue to be utilized as a source to generate viable *in vitro* models for neuroscience research. However, to recognise its full potential, the uncertainty regarding the cell types that arise in culture must be addressed. **METHODS**: This study optimized the culture environments and systematically classified the cell types that arise from cultures of the adult human brain by using immunocytochemical and electrophysiological techniques. **RESULTS**: Primary cultures contained $MAP2^+$ and $\beta III\text{-tubulin}^+$ neurons, $GFAP^+$ astrocytes, and $PU1^+$ and $CD45^+$ microglia. In addition, two mitotic populations with similar phenotypic characteristics were identified. They both possessed the ability to propagate in vitro and express neural progenitor cell (NPC) markers such as nestin and SOX-2. However, only one population could be differentiated into physiologically identifiable neurons and glia and therefore be classified as a NPC *in vitro*. The other population was classified as fibroblast-like cells (FbCs) due to co-expression of fibroblast-like markers. However, due to the FbC's tendency towards the neuroectodermal lineage, they may provide a readily available and reprogrammable cell source for neuroscience research. **CONCLUSION**: We anticipate that these results will further advance the field of adult human brain tissue culture and aid in the development of models that can recapitulate adult neurogenic processes. This system allows the key questions in brain plasticity to be asked in one of the most representative *in vitro* model of the adult human brain.

POS-WED-163

CD11B IS RAPIDLY INTERNALIZED IN ACTIVATED MICROGLIAL CELLS *IN VITRO*

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Purpose: Primary cultures of microglia are routinely used to study the properties of microglial cells *in vitro*. When stimulated, microglia transform from a resting into an activated state, characterized by morphological changes that can be visualized with antibodies targeting CD11b, an integrin receptor that plays a role in many cellular functions such as signalling and regulation of cell cycle. Activation of cultured microglia with bacterial lipopolysaccharide is associated with a sustained increase in intracellular Ca^{2+}. Adenosine triphosphate (ATP) is also thought to activate microglia, but the role of intracellular calcium in this activation is not clear. We therefore investigated the effect of ATP on intracellular calcium and trafficking of CD11b. **Methods:** Microglial cells were collected by shaking mixed glial cultures obtained from neonatal rat brains. Purity was determined to be >98% using immunocytochemistry for CD11b. Calcium imaging was performed using Fura-2-AM. Monoclonal OX42 antibody tagged with fluorescein was used to study the internalization of CD11b via 3D-confocal imaging. **Results:** In microglia with low initial levels of intracellular calcium, a one hour incubation with ATP caused a sustained increase in intracellular calcium in a dose dependent manner (EC_{50} = 10-50 µM, n≥5 at each concentration). In partially activated microglia with high initial intracellular calcium levels, fluorescently labelled antibodies to CD11b were internalized rapidly, but not localized to lysosomes after 6 hours (n≥5 cells per culture condition). **Conclusion:** ATP produces a long-term change in intracellular calcium at concentrations that would be expected to activate $P2X_4$ and not $P2X_7$ receptors. Increased intracellular calcium is associated with internalization of CD11b that may be required for microglial function.

POS-WED-164

DEVELOPMENT OF A HIGH CONTENT ASSAY TO QUANTIFY CANNABINOID-MEDIATED LEUKOCYTE ADHESION

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Aim: Cannabinoids improve neurological deficits in various rodent models of brain injury and degeneration, via activation of CB2 receptors expressed by immune cells. Our primary aim was to develop a non-invasive, high-content assay to investigate CB2 receptor regulation of leukocyte adhesion to brain cells. **Methods**: Peripheral blood mononuclear cells were isolated using a standard protocol from healthy volunteers (with Ethical Approval). The Discovery-1™ automated fluorescence microscope and Metamorph™ image analysis software were used to develop a robust, precise method to objectively quantify leukocyte adhesion. This method utilised nuclear size discrimination following Hoechst staining to differentiate between NT2 astrocyte nuclei (15-20µm) and that of adhering leukocytes (3-5µm). During optimisation, parameters were established for appropriate effector:target ratios, time-course of adhesion, acquisition (4-36 sites per well) and analysis for accurate quantification. **Results:** Leukocyte adhesion was observed under basal, non-inflammatory conditions and increased in the presence of TNF-α. Interestingly, in some experiments an increase (15-50%) in basal adhesion was observed following stimulation with the CB2 agonist HU308 (n=3 of 7). Preliminary analysis suggests that T-lymphocytes represent the majority of leukocytes adhering under these conditions. **Conclusions:** We have established a high content assay for measuring leukocyte adhesion suitable for investigating inflammatory and non-inflammatory events. CB2 activation increased leukocyte adhesion in some experiments. The variation in CB2 responsiveness is likely to reflect the variation in leukocyte phenotypes present. Surprisingly, no reduction was seen in basal leukocyte adhesion from any donor, which was initially expected given the proposed anti-inflammatory role of CB2 *in vivo*.

POS-WED-165

DEVELOPMENT OF *IN VITRO* MODELS OF HUMAN DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting from a loss of dopaminergic (DA) neurons in the *Substantia Nigra*. The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. The KP is activated in several neuroinflammatory diseases and is likely to be involved in PD pathogenesis. A prolonged activation of the KP leads to production and accumulation of the excitotoxin quinolinic acid (QUIN). We hypothesise that the KP in human DA neurons will produce neuroprotective KP metabolites and will be very sensitive to exogenous QUIN toxicity. **Purpose:** We aim to establish an *in vitro* model of human DA neurons for KP investigation. **Methods:** We have differentiated human neuroblastoma SH-SY5Y (n=3) and SK-N-SH (n=3) cell lines using 3 sets of treatments and characterised them for neuronal (MAP2) and DA marker (Tyrosine Hydroxylase -TH). For primary cells model, DA neurons were isolated from human foetal brain (16-20 weeks, n=3) by protocol established during this study. **Results:** Both cell lines treated with a combination of retinoic acid and BDNF in low serum conditions exhibited the DA neurons morphology. Though SH-SY5Y cell line expressed TH in higher levels compare to other cell line and treatments. Primary isolated cells can be maintained in culture for up to 4 weeks and express same neuronal markers. KP was induced in differentiated SH-SY5Y and isolated primary DA cells using INF-γ and then cells were characterised for set of KP genes using qPCR. **Conclusion:** These new and validated *in vitro* models will provide an important tool to study the involvement of QUIN in the death of DA neurons and also the neuroprotective ability of KP inhibitors as potential therapeutic for PD.

POS-WED-166

RNA EDITING AND ITS ROLE IN SYNAPTIC PLASTICITY AND COGNITION

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RNA editing by the ADAR (Adenosine Deaminases Acting on RNA) family of enzymes has contributed to the emergence of complex, higher organisms. In this study, we used a mouse model of learning and memory, to explore the interplay between regulatory RNA, RNA editing and the expansion of repetitive elements, within the context of plasticity related to cognitive function. Initial experiments at both in vitro and in vivo level show varied expression of ADARs (especially ADAR3) in response to KCl and fear conditioning, respectively and after extinction training. We are currently investigating the role of RNA editing in primary cortical neurons, in vitro, to elucidate how the different ADARs and their edited substrates respond in an activity-dependent manner. Our future goal is to establish the role of RNA editing in associative learning, and to detail the influence of RNA editing events during the formation of long-term memory. The evolution, prevalence and environmental adaptation of RNA editing provide compelling evidence that this mechanism of genetic modification is indispensible for higher order cognition in the mammalian brain.