POSTERS

POS-MON-001

THE EFFECTS OF SUBSTRATE (MATRIX) COMPOSITION ON THE DIFFERENTIATION OF NEURAL STEM CELLS INTO DOPAMINERGIC NEURONS

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Purpose: As research in the field of neural stem cells (NSCs) continues the production of dopaminergic neurons from NSCs may be of benefit to both the pharmaceutical industry and basic research scientists. We have investigated the effects of laminin on the differentiation of NSCs *in vitro* by using functional and immunocytochemistry experiments. **Methods:** NSCs were grown on 24-well plates (n=32), with and without laminin, and exposed to various combinations of morphogens [brain-derived neurotrophic factor (10 ng/mL), glial cell line-derived neurotrophic factor (10 ng/mL) and ascorbic acid (200 μM)]. Cells were checked for functionality on days 15 & 22 by measuring the effect of the agonists noradrenaline (30 μM), acetylcholine (30 μM), L-glutamine (30 μM) and ATP (300 μM) on intracellular calcium. Immunocytochemistry studies examined the extent of differentiation of the NSCs. **Results and Conclusions:** Immunocytochemistry showed that in the presence of laminin about 5 % of the total cell population differentiated into tyrosine hydroxylase positive, 'dopaminergic' neurons. Still more experiments are needed to be carried out to elucidate the role of laminin in the differentiation and development of neurons derived from NSCs.

POS-MON-002

THE CELL ADHESION MOLECULE, L1, IS INVOLVED IN THE DIFFERENTIATION OF ENTERIC NEURONS IN THE DEVELOPING GUT

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The enteric nervous system is comprised of many different functional subtypes of enteric neurons. During development, the enteric nervous system arises predominantly from neural crest cells that migrate from the caudal hindbrain (vagal level) into and along the developing gut. As vagal neural crest cells migrate within the gut, a subpopulation begins to differentiate into enteric neurons. However, little is known about the factors that influence enteric neural crest cell differentiation. Recently, the cell adhesion molecule, L1, has been shown to promote neuronal differentiation and inhibit glial cell differentiation of neural precursors in vitro (Dihne et al., 2003). In addition, L1 was shown to modify the neurotransmitter-specific subtype of neurons generated from the neural precursors (Dihne et al., 2003). Enteric neural crest cells express L1 as they migrate within the developing gut. This raises the possibility that L1 may play a role in the differentiation of neural crest cells into enteric neurons and glial cells. In this study, L1-deficient mice were used to examine whether there were any genotype-specific changes in the differentiation of enteric neurons and glial cells in the gut of E13.5-18.5 embryos. Neuronal differentiation was found to be delayed in L1-deficient mice compared to littermate controls (n>6). In addition, the onset of a specific subtype of enteric neuron was also found to be delayed in L1-deficient mice (n>6). Together, these results demonstrate a role for the cell adhesion molecule, L1, in the differentiation of enteric neurons. Dihne M, Bernreuther C, Sibbe M, Paulus W, Schachner M. (2003). J Neurosci. 23:6638-50.

POS-MON-003

NETRIN RECEPTORS AND THEIR LIGANDS: GUIDING MIGRATION IN THE ADULT BRAIN

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Purpose: While the ability of the adult brain to produce new neurons is now well established, the molecular mechanisms guiding migration of these new neurons from a proliferative zone to their final destination are still poorly understood. The Netrin family of guidance cues and their receptors have a known role in neuronal and axonal guidance in the embryo. A suitable model to study the function of these molecules in migration in the adult mammalian brain is the rostral migratory stream (RMS), as this is the path used by neuronal precursors to migrate from the subventricular zone to the olfactory bulb (OB) throughout life. **Method:** Expression studies were conducted on frozen sagittal sections of mice aged 8–12 weeks. The expression pattern of Netrin receptors in the RMS was analysed using antibodies specific to receptors and cell maturation markers in the C57Bl/6 forebrain (n=3). Netrin expression was inferred from LacZ staining in the Netrin-1 mutant forebrain (n=2). **Results:** Our experiments have shown that Neogenin and DCC are found on migrating neuroblasts throughout the RMS. Further, while Netrin-1 is not expressed in the OB, we have found it is expressed in the rostral brain ventral to the RMS. **Conclusion:** Together, these data suggest the Netrin receptors and their ligands are potential guidance molecules for migration along the adult RMS. We are currently studying which molecules are relevant to neuroblast migration along the RMS and the complex relationship between these molecules.

POS-MON-004

PURINERGIC SIGNALLING REGULATES INTERNEURON MIGRATION IN THE DEVELOPING CORTEX

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Purpose: Neuronal migration is one of the critical features in the construction of the mammalian neocortex. It is well-established that cortical interneurons originate from the ganglionic eminence of the ventral telencephalon and migrate tangentially during early corticogenesis. How these neurons migrate within the neocortical primordium and what factors influence this process still remains unclear. **Method**: Real-time confocal imaging of embryonic brain slice cultures from glutamate decarboxylase-(GAD)-67 GFP knock-in mice. Images taken at 5 min intervals over a 240 min period were animated and analyzed using AutoTrace, a MATLAB based application. **Results**: Our imaging revealed various directions of migration undertaken by pioneer interneurons entering the neocortex at embryonic day E12-E13. Although 40% of interneurons undergo tangential migration (n=17) parallel to the the lateral-medial axis, a significant number, 60%, migrate radially towards the pial surface, or radially towards the ventricular zone (n=28). Further examination revealed that certain populations display synchronized somal movement with uniform periodicity. This periodicity is dependent on the direction of migration and the presence of extracellular calcium. We further characterized the participation of ATP signalling in this process and show that the radial migration towards the ventricular zone is sensitive to the P2 receptor blockers Suramin and PPADS (n=54) and to the extracellular ATP degrading enzyme Apyrase (n=26). **Conclusion**: Synchronized somal translocation and multiple modes of migration within the interneuron population indicate that both intrinsic and extrinsic factors play a role in controlling the rate and direction of neuronal migration.

POS-MON-005

THE NETRIN RECEPTOR, NEOGENIN, PLAYS A ROLE IN MIGRATION IN THE DEVELOPING EMBRYONIC FOREBRAIN

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Purpose: At the onset of neurogenesis, vigorous proliferation occurs within the ventricular zones of the forebrain. The neuronal progenitors of the cortex are the radial glia, which give rise to pyramidal neurons that then migrate towards the outer surface. In the ventral forebrain, progenitors give rise to interneurons that migrate tangentially into the developing cortex and integrate into the circuitry of the brain. Abnormal neuronal migration is the underlying cause of several human disorders, including lissencephaly and epilepsy. Neogenin has been identified as a receptor for members of the Netrin family, which play a pivotal role in the guidance of young neurons and axonal projections during embryonic development. It has been proposed that Neogenin may be important in a range of cellular processes, including proliferation, migration, and axon guidance (Rajagopalan et al., 2004; Cole, Bradford & Cooper, 2006). **Methods:** We examined embryonic mice (n=5) using immunohistochemical techniques to investigate Neogenin expression during peak neurogenesis. **Results:** We have demonstrated that at embryonic day 12 to 14, Neogenin is expressed on neural progenitors within the ventricular zones of the cortex and lateral ganglionic eminence. Furthermore, Neogenin expression can also be found on young interneurons as they migrate from the ganglionic eminences in the ventral forebrain into the cortical plate. These include the parvalbumin- and calbindin-expressing interneuron subpopulations. **Conclusion:** Our preliminary data suggests that Neogenin may play a role in the migration of these interneurons within the ventral forebrain. Further work will explore the precise role of Neogenin in interneuron migration during mammalian forebrain development.

POS-MON-006

TIME-LAPSE IMAGING OF BEHAVIOUR OF EARLY ENTERIC NEURONS

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Enteric neurons arise from precursors originating in the neural crest that migrate into and along the developing gut. During migration, a sub-population (around 10-20%) of cells start to express neuron-specific markers, including transient expression of the catecholamine synthetic enzyme, tyrosine hydroxylase (TH). The migratory behaviour of these immature enteric neurons has not been previously examined. Of particular interest is whether early differentiating neurons are capable of migrating, or whether enteric crest-derived cells cease migrating as soon as they commence neuronal differentiation. We used embryonic TH-GFP mice, and first confirmed that GFP+ cells in the gut also express the pan-neuronal markers TuJ1 and neurofilament-M. Explants of gut were set up for time lapse imaging (n = 80). 57.5% of the GFP+ neurons were defined as stationary as their cell bodies did not change location during the imaging period. However they still showed dynamic behaviour as they extended and retracted processes, and their cell bodies often underwent significant changes in shape. Around 42.5% of the GFP+ neurons did migrate, at a mean speed of 11 μm/h. The mode of migration of these neurons was different from that of undifferentiated neural crest cells. The GFP+ neurons usually had a prominent leading process, with the cell body at the rear. A swelling was often observed to precede the cell body, which may be the centrosome, or microtubule organizing centre, of the cell. These data show that, like some neuron populations in the developing CNS, some immature enteric neurons in the embryonic gut are capable of migration.

POS-MON-007

RYK IS A NOVEL WNT RECEPTOR REQUIRED FOR AXON GUIDANCE BEFORE AND AFTER MIDLINE CROSSING IN THE CORPUS CALLOSUM

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Purpose: Ryk has been shown to be a novel Wnt receptor in both invertebrates and vertebrates. We recently reported that in Ryk-deficient mice, cortical axons project aberrantly across the major forebrain commissure - the corpus callosum. On the C57Bl/6x129sv background, loss of Ryk does not interfere with the ability of callosal axons to cross the midline but impedes their escape from the midline where they form axon bundles rather than projecting into the contralateral hemisphere. We report here that loss of Ryk on a pure 129sv background results in more severe callosal guidance defects. **Methods:** Embryonic day 18 *Ryk+/+* (n=5), *Ryk+/-* (n=14) and *Ryk-/-* (n=9) embryos on the 129sv background were perfusion fixed with 4% paraformaldehyde. Haematoxylin and eosin histological staining was performed on 5μm coronal sections cut on a microtome from paraffin embedded tissue. Immunostaining with antibodies against L1 CAM, an axon-specific cell adhesion molecule, was performed on 50μm coronal sections cut on a vibratome. **Results:** The previously described 'Ryk phenotype' was observed in 11% of *Ryk-/-* and 14% of *Ryk+/-* embryos on the pure 129sv background. In addition, 78% of *Ryk-/-* and 36% of *Ryk+/-* embryos displayed an acallosal Probst bundle phenotype, where labeled axons successfully reached the midline but failed to cross it. **Conclusions:** We show that Ryk signaling is also required for callosal guidance across the midline. However, this activity is tightly regulated and dependent on modifier genes present on these genetic backgrounds.

POS-MON-008

TROPOMYOSINS IN DYNAMIC STRUCTURES OF NEURONAL CELLS

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During development neurons depend on a dynamic cytoskeleton in order to establish a complex cellular architecture. Tropomyosins constitute a family of proteins that define distinct pools of actin filament populations, a major component of the cytoskeleton. Tropomyosins are encoded by four different genes and stabilize microfilaments by binding along their major groove. Aim of this study is to analyze the role of γTm gene products by using an exon 9d specific gene knockout mouse model in which two tropomyosin isoforms are eliminated, Tm5NM1 and Tm5NM2. The reduction of exon9d-containing isoforms is compensated by an upregulation of exon 9c-containing isoforms. Effects of the lack of Tm5NM1/2 in cultured primary neurons were analyzed at early stages of development. The loss of Tm5NM1/2 leads to only minor changes in growth cone size, retraction rates and extension rates of growth cone lamellipodia. The results are consistent with earlier observations that showing that the loss of Tropomyosins can be compensated by the cell through upregulation of alternatively spliced products of the same gene. The compensatory mechanism appears to be sufficient to maintain basic properties of growth cones in neuons and the presence of Tm5NM1/2 is not essential for proper neuronal development. Further studies will aim to analyze the impact of changed levels of Tropomyosin isoforms from the γ- as well as the δ-Tm gene on growth cones and dendritic spines. This will give us a better understanding of actin-cytoskeleton related processe which underlie neuronal morphogenesis and function.

POS-MON-009

INVOLVEMENT OF EPHA4 RECEPTOR TYROSINE KINASE IN ASTROCYTE EXTRACELLULAR MATRIX ADHESION AND RHO MEDIATED CYTOSKELETAL REGULATION

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Spinal cord injury in EphA4 null mice revealed functional recovery and improved behavioural outcome compared to wildtype animals. This appeared to involve a lack of robust astrocytic gliosis, with only a modest increase in GFAP expression in EphA4 null animals (Goldshmit et al. J. Neurosci. 2004). How EphA4 regulates cytoskeletal changes occurring after astrocytic activation and whether these influence adhesion is not yet known. We investigated effects of EphA4 signaling on cytoskeletal rearrangement and adhesion of astrocytes in vitro. Regulation of the F-actin cytoskeleton was examined by inducing cytoskeletal collapse with the Rho kinase (ROCK) inhibitor HA1077 (0.1 mM), followed by recovery of stress fibre formation after HA1077 washout. This was examined by F-actin staining with phalloidin-FITC with (n=6) and without (n=8) activation of EphA4 with ephrinA5-Fc. Under basal conditions, no significant differences between genotypes in percentage of cells containing stress fibres (approximately 85%) were detected. Without EphA4 activation, 15 minutes after HA1077 removal 47% of wildtype astrocytes and 40% of EphA4 null astrocytes re-established stress fibres. However, 30 minutes after HA1077 removal the percentage of cells expressing stress fibres was back to basal levels in both genotypes. EphA4 activation enhanced recovery of stress fibre formation and was further increased in EphA4 null cells, indicating activation of another Eph receptor. These results suggest an intrinsic difference between genotypes in regulation of the F-actin cytoskeleton mediated by EphA4. To investigate involvement of EphA4 in astrocyte adhesion, the ability of astrocytes to adhere to laminin, poly-D-lysine or uncoated surfaces was examined (n=5). Wildtype astrocytes showed significantly higher adhesion on these substrates suggesting a role for EphA4 in focal adhesion and integrin regulation.

POS-MON-010

SYNAPTOGENESIS AND STRATIFICATION IN THE MUSHROOM BODIES OF THE HONEYBEE

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Purpose. Stratification is a common anatomical feature of central brain in both vertebrates and invertebrates. Complex stratification of the insect mushroom bodies (MBs), higher associative centres involved in memory formation, is believed to reflect segregation of functionally distinctive populations of the MB neurons. During development, the MB strata are sequentially added resulting in a birthdate-dependent arrangement of the MB neuron axons in the peduncle and lobes. The aim of this study was to investigate ultrastructural correlate of fine stratification as well as relationships between fine stratification and synaptogenesis in developing MBs of the honeybee. **Methods**. Pupal stages P1-P9 (N=18) were examined by means of combined light and electron microscopy. **Results**. During metamorphosis, the MB vertical lobe showed progressive stratification with thick strata and thin laminae. However, lamination pattern was not consistent along axis of the vertical lobe, indicating that new laminae appeared within the lobe rather than were sequentially added. Most dark laminae or strata were composed of tightly packed axons of the MB intrinsic neurons, while light laminae or strata represented differentiating synaptic neuropile with higher density of synaptic appositions. **Conclusion**. Synaptogenesis within dark laminae or strata occurs with a delay relative to those in light laminae or strata, and this delay appears to be independent from the birth sequence of the MB neurons. Such a local transient block of synaptogenesis may provide correct targeting subsets of the MB neurons by specific extrinsic MB neurons.

POS-MON-011

DIFFUSION TENSOR IMAGING AND TRACTOGRAPHY OF DEVELOPING MOUSE FOREBRAIN COMMISSURES

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Diffusion tensor imaging (DTI) is a sensitive tool for visualizing ordered structures in brain tissue such as axon tracts such as the forebrain commissures (anterior commissure, hippocampal commissure and corpus callosum). **Purpose:** To utilise DTI and tractography to investigate the relative development of the three forebrain commissures. **Methods:** We used high-resolution DTI of fixed mouse brains from E14 to adult (n=3) to study developmental changes in regional diffusion anisotropy and white matter fibre tract development. Imaging was performed on an 11-tesla magnetic resonance system using solenoid coils. Fractional anisotropy maps and fibre tracts were generated using DTI Studio software and compared across different embryonic and postnatal ages. A novel oblique coronal plane was utilised for comparison of the relative position of individual commissures crossing the midline. **Results:** The brain and white matter FA values increased with embryonic age and were highest at birth (~200-300% of E16). After birth, white matter FA intensities dropped markedly to just above those values seen at E16. However, from P15 onwards a gradual increase in FA intensity (~120% of pre-P15 intensities) was noticed in all commissures, which correlates with axonal myelination. Region of interest (ROI) DTI tractography of the corpus callosum showed a rostral to caudal expansion of interhemisphere tracts, in agreement with previous histological analyses. Hippocampal and anterior commissures were seen to cross the midline by E16, which by this stage were distinct from those of the corpus callosum. **Conclusion:** Ex vivo DTI tractography is a powerful tool for examining gross or subtle changes in commissure fibre development. Such studies may prove important for understanding human commissural brain malformations.

POS-MON-012

CORPUS CALLOSUM DEVELOPMENT IN THE PRETERM INFANT: AN MRI STUDY

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Purpose: To determine the difference between corpus callosum (CC) development in preterm (PT) and full term (FT) infants utilizing structural and diffusion MRI. **Methods:** MRI was performed at term equivalent with a 1.5T GE scanner, utilizing line scan diffusion and structural 3-D T1 spoiled gradient recalled and T2 dual echo (t2w and pdw) fast spin echo sequences. T1 weighted scans were transformed into standard space along the AC-PC line. The CC was then traced on the mid-sagittal slice of PT (n=10) and FT infants (n=10), chosen as the first of a cohort of 173 infants' diffusion MRI scans obtained at the Royal Women's Hospital, Melbourne. Probablilistic tractography was performed on regions of interest placed on the genu and splenium of the CC in the diffusion anisotropy images of the 20 subjects. Estimation of the fractional anisotropy and connectivity of the resulting white matter fibre tracts of the CC was calculated. **Results:** Preterm infants demonstrated significantly reduced CC volumes on the mid-sagittal slice (PT: mean (SD) 0.115 (0.02); FT: 0.134 (0.02) cm^3; p=0.049). Fibre connectivity was significantly reduced in the genu (PT 1861 (487); FT: 2296 (348) mm^3; p=0.03), but not the splenium (p=0.3) of the CC in PT infants. Fractional anisotropy values were not sigificantly different between PT and FT infants for either the genu or splenium of the CC (p>0.7). **Conclusion:** Preterm infants have altered corpus callosum structure and connectivity when compared to full term infants at term equivalent age, which may reflect delayed development, especially in the anterior portions of this structure.

POS-MON-013

SUBREGIONS WITHIN THE MOUSE NEOSTRIATUM EXHIBIT DIFFERENTIAL PATTERNS OF MATURATION

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Purpose: Cortico-striatal circuits within the mammalian brain have been implicated in the execution of volitional action as well as the selection of motor programs. Control and refinement of motor behaviors are thought to come "on-line" during early development, but how these changes occur is not known. The aim of this study is to reveal anatomical correlates of this maturation by examining the distribution of chondroitin sulfate proteoglycans (CSPGs) (a marker for plasticity) with respect to the striosome/matrix sub-compartments in the developing mouse neostriatum, the input nucleus of the basal ganglia. **Methods:** Coronal sections of mouse brains of different ages (postnatal days 4, 10, 14, 21, 26~28, >40, n=3 per age grouping) were double labeled with Wisteria Floribunda Agglutinin (WFA) to visualize CSPGs, and μ-opioid receptor (MOR1) antibody to differentiate striosome/matrix sub-compartments. **Results:** Neostriatal sub-compartments exhibited noticeable differences in their pattern of CSPG expression during early development. WFA labeling revealed that in neonates (P4), CSPGs were limited to cloud-like patches overlapping a subset of the MOR1 reactive striosomes. This expression pattern changed, however, with striosomes becoming devoid of CSPG expression by P21. In contrast, CSPG labeling was first detected in a subregion of the matrix at P10, in the form of perineuronal nets (PNN). Formation of these CSPG structures continued to expand throughout the neostriatum, with the entire matrix expressing PNNs at later developmental stages (P21 onwards). **Conclusion:** This study demonstrates the presence of differential CSPG expression in the striosome/matrix sub-compartments during development. These findings suggest that these distinct neostriatal structures may serve unique roles in the maturation of cortico-striatal circuits vital for self-initiated action and the refinement of motor function.

POS-MON-014

DEVELOPMENTAL VITAMIN D DEFICIENCY ALTERS THE TRAJECTORY OF BRAIN GROWTH BUT NOT THE RESPONSE TO PSYCHOMIMETICS IN C57BL/6J MICE

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Introduction: Developmental vitamin D (DVD) deficiency has been proposed as a risk factor for several brain disorders. DVD-deficiency alters neuroanatomy and increases locomotor sensitivity to psychomimetics in rats. DVD-deficiency has also been shown to alter exploratory behaviour in mice. The aim of this study was to investigate the effect of DVD-deficiency on neuroanatomy in neonatal and adult offspring and psychomimetic-induced locomotion in adult offspring. **Methods:** Female C57BL/6J mice were fed a vitamin D-deficient diet from 6-weeks prior to conception until birth, and transferred to a diet containing vitamin D. Control mice were fed a normal diet throughout the experiment. Neuroanatomy was investigated in newborn (n=9) and adult mice (n=4). Locomotor sensitivity to 5mg/kg d-amphetamine, 0.5mg/kg MK-801 or saline was examined in an open field (n>15). **Results:** Neonatal DVD-deficient mice had a significant increase (15%; $P<0.01$) in hippocampal volume with no change in brain volume. Pilot studies in adult males revealed a decrease in the brain volume of DVD-deficient mice (32%; $P<0.01$). Maternal diet did not affect the size of internal structures when corrected for brain size. There was no significant effect of maternal diet on amphetamine or MK-801 induced locomotion. **Conclusions:** These data suggest that DVD-deficiency alters the trajectory of brain development in C57BL/6J mice. Despite the substantial reduction in brain volume (32%), behavioural results imply dopaminergic and glutamatergic systems in the adult are intact. Pharmacological studies are now being conducted in 129/SvJ mice as to better understand the interactions between DVD-deficiency and genetic background that underlie psychomimetic responses.

POS-MON-015

$GABA_A$ RECEPTOR SUBUNIT EXPRESSION IN NORMALLY GROWN AND IUGR PIGLET BRAIN

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Intrauterine growth restriction (IUGR) is a major cause of perinatal mortality and neuromorbidity; seizure risk in the newborn brain is further compounded by IUGR (McIntire et al. 1999). Prenatal protein malnutrition significantly impacts on fetal growth and development (Durosseau et al. 2003). In adult rat models, prenatal malnutrition alters expression levels of $GABA_A$ receptor α_1, β_2 and α_3 mRNA (Steiger et al. 2003). Aberrant mRNA expression of $GABA_A$ subunits expressed in the developing brain may underpin epileptogenesis (Poulter et al. 1999). Changes in $GABA_A$ receptor protein expression levels have not been examined. Purpose: To compare the protein expression level of the $GABA_A$ receptor α_1, α_3 and β_2 subunits at P0 and P7 in normally grown (NG) and IUGR piglets. Methods: IUGR piglets were born spontaneously. Animals were euthanased at P0 (NG n=6, IUGR n=6) and P7 (NG n=8, IUGR n=8) and tissue collected from parietal cortex and hippocampus. Western blotting was used to detect subunit protein expression levels. Results: $GABA_A$ receptor α_3 protein expression was significantly increased in P7 IUGR cortex whilst β_2 protein expression was significantly decreased in cortex of P0 IUGR animals. $GABA_A$ receptor α_3 protein expression relative to α_1 protein expression was significantly greater in cortex of P0 NG animals. No changes were observed in the hippocampus or in α_1 expression. Conclusion: Expression levels of α_3 and β_2 proteins were significantly altered in IUGR piglet cortex at different ages. Differences in $GABA_A$ receptor subunit expression in IUGR animals may give rise to greater vulnerability to brain injury and seizures.

POS-MON-016

THE EVOLUTION OF MYELIN PROTEIN SIGNALLING

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Introduction: Axonal regeneration is inhibited in the CNS after injury. Three molecules responsible for this inhibition are Nogo, Myelin Associated Glycoprotein (MAG) and Oligodendrocyte Myelin Glycoprotein (OMgP). These molecules signal through a tri-receptor complex comprised of Nogo receptor (NgR), p75 neurotrophin receptor (p75) and LINGO-1. The activation of RhoA has been implicated in this process. Interestingly, studies in the p75 knockout mice have shown that neurite inhibition persists in the presence of these myelin inhibitors, suggesting receptor redundancy. **Purpose:** The Neurotrophin Receptor Homologue-2 (NRH2) is a recently identified gene that has high sequence similarity to p75. Evolutionary analysis suggests that NRH1 was formed from a p75 gene duplication event at the emergence of the vertebrate lineage. At the evolutionary divergence of mammals, the NRH1 gene underwent a mutation that truncated the extracellular domain, resulting in the formation of NRH2. We sought to investigate whether NRH could functionally replace p75 in myelin signalling. **Methods:** To test this hypothesis, we performed immunoprecipitation experiments of the tri-receptor complex in the presence of p75, NRH1 and NRH2. **Results:** p75 interacted with both NgR and LINGO-1. While an association between *Xenopus* NRH1, NgR and LINGO-1 could be detected (n=3), no such interactions were formed with NRH2 (n=3). Further, we identified that the extracellular domain of p75 is necessary and sufficient to form this receptor complex (n=3). **Conclusion:** Our data indicate that NgR and LINGO-1 forms an interaction with *Xenopus* NRH1, but not with mammalian NRH2, suggesting a functional distinction in myelin signalling between early vertebrate and mammalian lineages. We are currently investigating whether a NRH1/NgR/LINGO-1 tri-receptor complex activates RhoA and inhibit neurite outgrowth.

POS-MON-017

MYELINATION POTENTIAL OF OLFACTORY ENSHEATHING GLIA: A COMPARISON WITH SCHWANN CELLS

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Purpose: A crucial aspect of CNS repair is the remyelination of axons resulting in restoration of functional conductivity. Transplanted olfactory ensheathing glia (OEG) are reported to provide this role in the damaged CNS in a similar manner to grafted or migratory Schwann cells (SC). Methods: Using immunohistochemistry, western blots (WBs) and quantitative real-time PCR, we examined basal protein and mRNA levels of protein zero (Po), myelin basic protein (MBP), myelin associated glycoprotein (MAG) and 2'3'-cyclic nucleotide 3' phosphodiesterase (CNP) within the olfactory bulb (n=4), p75 purified bulb cultures of adult OEG (n=16) and adult SC cultures (n=12), in the presence of known myelin inducing factors eg cAMP. Results: Comparative in vitro analysis of OEG and SCs showed increased Po protein expression in OEG when cAMP was present, but levels were reduced significantly by the addition of serum. SCs grown in serum-free media supplemented with neuregulin β1 (NRG1) expressed Po, but no Po was observed in OEG under these conditions. In all growth media, basal expression of MAG or MBP in OEG was not detected by WBs. CNP protein was present in OEG and in SCs in all tested media, however CNP levels were lower in OEG especially in the presence of cAMP. PCR analysis of OEG revealed expression of mRNAs for all myelin-related genes, the levels regulated by NRG1 and cAMP. Conclusions: OEG have some non SC-like myelin gene expression characteristics in vitro. These differences suggest separate regulatory mechanisms of myelin expression and may indicate an inability of OEG to form compact myelin.

POS-MON-018

BRAIN DERIVED NEUROTROPHIC FACTOR MEDIATES CENTRAL MYELINATION VIA TRKB RECEPTOR SIGNALLING

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Myelin formation requires complex and dynamic signalling between neurons and oligodendendrocytes in the central nervous system (CNS). Loss of myelin and the subsequent failure of remyelination by oligodendrocytes contribute to the functional impairment that characterizes demyelinating disease such as Multiple Sclerosis. Brain Derived Neurotrophic Factor (BDNF) has recently shown to promote peripheral myelination during development and remyelination after injury. However the roles that BDNF exerts on central myelination are unknown. **Purpose and methods**: To investigate the role that BDNF plays in controlling central myelination and its interrogate downstream signalling pathways BDNF activated, we use *in vitro* myelination assays co-culturing dorsal root ganglia neurons (DRG) with oligodendrocyte precursor cells (OPC). Myelination was assessed via fluorescent immunocytochemistry and by western blot of co-culture lysates. **Results**: In the DRG-OPC co-cultures, exogenous BDNF significantly enhanced myelination by oligodendrocytes. Our data shows that the BDNF receptors TrkB and p75NTR are expressed dynamically during both the oligodendrocyte lineage and myelination in vitro. We found that TrkB receptors and the downstream MAPK/ERK pathway are activated by BDNF in myelination co-cultures. Furthermore, blocking TrkB receptor activation using a TrkB blocking peptide or the tyrosine kinase inhibitor K252a both dramatically reduced the myelin development, indicating that TrkB receptors mediate the effect of BDNF on central myelination. **Conclusion**: Our data suggested that BDNF enhances central myelination via direct activation of TrkB receptors expressed by oligodendrocytes.

POS-MON-019

THE EMERGING ROLE OF PRONEUROTROPHIN SIGNALLING IN TRANSCRIPTIONAL REGULATION AND MYELINATION BY SCHWANN CELLS

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We are investigating the molecular signals that regulate myelination within the peripheral nervous system. Previous data suggest that the mature neurotrophins exert important influences, with nerve growth factor (NGF) promoting peripheral myelination but inhibiting central myelination, brain derived neurotrophic factor (BDNF) promoting myelination, whereas a third member neurotrophin-3 inhibits it. It is also reported that activation of the transcription factor NFκB, which is known to be regulated in Schwann cells by mature NGF, is essential for Schwann cell maturation and progression to a myelinating phenotype. Recently it has been reported that the precursor or pro forms of BDNF and NGF are biologically active. They signal through a receptor complex comprising of the p75 Neurotrophin Receptor (p75NTR) and Sortilin, and exert distinct biological effects to their mature forms. Here we identify that p75NTR and Sortilin receptors are expressed in Schwann cells, DRG neurons and oligodendrocytes. Utilising in vitro luciferase reporter assays (n=4), we identify that both pro-NGF and pro-BDNF significantly increase activation of NFκB over that seen with the mature forms. Our data indicate that pro-NGF and pro-BDNF have no significant effect on Schwann cell proliferation, differentiation or survival in vitro (n=4). Taken together, this suggests a potential role for pro-NGF and pro-BDNF in promoting Schwann cell myelination. Utilising in vitro myelination assays, we are currently addressing this question, investigating the role that these factors play in regulating Schwann cell myelination. These experiments will determine whether the conversion of the pro-neurotrophins into their mature form differentially regulates Schwann cell myelination.

POS-MON-020

THE RABBIT RETINA FACILITATES THE IDENTIFICATION OF A NEW CELL IN VIVO

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Purpose: The myelinated streak (MS) of the rabbit retina is comprised of the same cells as the rat optic nerve (RON). The RON is commonly used to characterise glia in the Central Nervous System (CNS), however the compressed nature of this tissue makes it difficult to study astrocytes *in situ*. The MS of the rabbit retina offers a model system to study heterogeneity of astrocytes in intact CNS tissue and their interaction with blood vessels. **Methods:** Triple-label immunohistochemistry was used to identify different sub-populations of astrocytes in the MS of the rabbit retina (n=34). Antibodies against O4, GFAP, S100β, connexin-43, glutamine synthetase (GS), ezrin, nestin, βIII tubulin and Map 1b were used to investigate cells in the MS according to their antigenic properties. The expression of NG2, SMA and desmin were used to visualise pericytes and smooth muscle cells on blood vessels. **Results:** Sub-populations of astrocytes were identified based on differences in developmental appearance, contact spacing and antigenic expression. O4- astrocytes representing the majority of these cells differentiated at P0, displayed contact spacing and expressed GFAP, S100β, GS and Connexin 43. A smaller population of astrocytes did not express Connexin 43, lacked contact spacing, differentiated at P5-P7 and demonstrated a close association with blood vessels. Cells expressing both O4 and Map 1b have also been found within the MS. **Conclusion:** We have provided developmental, morphological and functional evidence of two distinct populations of astrocytes in intact adult CNS tissue. The O4+ astrocyte has not previously been characterised *in vivo*. Consistent with earlier reports, the O4+ astrocytes identified in this study are thought to be the *in vivo* equivalent of the type 2 astrocyte previously described in tissue culture. The interaction of O4+ astrocytes with Desmin and SMA filaments also suggests a distinct role that these cells play relating to the regulation of blood flow.

POS-MON-021

OLFACTORY ENSHEATHING CELLS ARE ATTRACTED TO, AND ARE CAPABLE OF PHAGOCYTOSING BACTERIA

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Purpose: This project investigates whether olfactory ensheathing cells demonstrate chemotaxis towards bacteria and are able to eliminate them by phagocytoses and lysosomal digestion. **Methods:** To demonstate chemostaxis, a pseudopodia assay was performed. Olfactory ensheathing cells were isolated from neonatal hooded Wistar rats and cultured on polycarbonate membrane Transwell inserts of 12 µm pore size and 100 µL of test solution (with or without Escherichia coli) was added to the chamber beneath. LysoTracker is a red fluorescence probe that accumulates selectively in cellular compartments such as lysosomes which have a low internal pH. We used this probe to determine whether FITC-conjugated *E. coli* internalised by olfactory ensheathing cells were translocated to lysosomes. Transmission electron microscopy was performed to confirm the fate of endocytosed *E. coli* . **Results:** Based on results from 4 separate experiments, it was shown that significantly more pseudopodia extended through the pores when the test solution contained *E. coli* (p=0.02). LysoTracker revealed that exposure to *E. coli* induced the formation of more lysosomes in olfactory ensheathing cells and that internalized fluorescent *E. coli* co-localised with the lysosomes. Transmission electron microscopy showed that many *E. coli* adhered to the surface of olfactory ensheathing cells and that *E. coli* at various stages of degradation were present in the lysosomal vesicles. No obvious adherence to the membrane and less phagocytosis was observed when olfactory ensheathing cells were incubated with fluorescent microspheres. **Conclusion:** Olfactory ensheathing cells are attracted to and are capable of phagocytosing *E. coli* .

POS-MON-022

INDUCIBLE PRODUCTION OF NITRIC OXIDE BY OLFACTORY ENSHEATHING CELLS IN RESPONSE TO BACTERIA

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The olfactory pathway represents a route for pathogens to access the central nervous system from the nasal cavity. Olfactory ensheathing cells (OECs), glial cells which ensheath the olfactory nerves are in a prime position to assist with host immunity. **Purpose:** This project investigates possible mechanisms relating to OEC's hypothesised role in host immunity, including the production of nitric oxide (NO), a potent antibacterial and antiviral agent. **Methods:** OECs were incubated with *Escherichia coli* and *Staphylococcus aureus*. Nitrite and NO production were analyzed using HPLC and live cell imaging respectively, mRNA levels by RT-PCR, and iNOS expression by immunocytochemistry (all n=3). To examine the *in vivo* expression of the chemokine receptor CX_3CR1 and iNOS in OECs, $CX_3CR1^{+/GFP}$ mice were administered with *S.aureus* fluorescent Bioparticles or PBS for 3, 6 and 24 hours, then perfused, fixed,decalcified, cryoprotected, cryostat sectioned and immunostained for iNOS (n=3). **Results:** We show that bacteria-treated OECs produced elevated levels of NO using DAF2-DA which was attenuated by the NO synthase inhibitor L-NMMA. Expression of iNOS was elevated in bacteria-incubated OECs compared to untreated OECs. Elevated levels of nitrite were detected in bacteria-treated OECs (also attenuated by L-NMMA) compared to untreated OECs. mRNA was detected for iNOS in OECs but not for nNOS or eNOS. **Conclusions:** Bacteria-treated OECs produce NO and express iNOS*in vitro*. Preliminary *in vivo* studies indicate that subsets of OECs express CX_3CR1 and iNOS, but these are not upregulated in response to bacteria in the uncompromised epithelium.

POS-MON-023

TRANSFER ACROSS CHOROID PLEXUS DURING BRAIN DEVELOPMENT

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Purpose: The choroid plexuses within the ventricles of the brain are comprised of epithelial cells involved in cerebrospinal fluid (CSF) secretion and transfer of molecules from blood into CSF. Due to the presence of tight junctions between choroidal epithelial cells, the route of transfer is suggested to be transcellular. (Ek et al., 2006). In the present study, routes of transfer for small and large molecules were compared in the same animal model. **Methods:** *Monodelphis domestica* pups at several ages (n = 3 at each age) were injected with biotinylated dextrans (3kDa, 10kDa, 70kDa). CSF, blood and brains were collected from terminally anaesthetised animals. Brains were processed for histology. 5µm coronal sections were stained for a range of individual plasma proteins and to detect biotinylated probes. Protein positive cells were counted. Fluorescent staining was used to co-localise individual proteins and biotinylated probes. Total and individual protein concentrations in CSF and plasma were determined by Bradford protein assay and western blot analysis. **Results:** Numbers of protein positive cells increased during development, in line with the concentration of protein in plasma. The percentage of total choroid plexus cells positive for protein remained constant. The number of cells positive for biotinylated probes increased with age, but their percentage of total cells decreased. Co-localisation of different proteins showed specificity for individual proteins in some epithelial cells. Endogenous proteins and biotinylated probes co-localised in about 10% of cells. **Conclusion:** This data indicate that two transfer mechanisms are present from very early in development, suggesting that the blood-CSF barrier is functionally mature from the earliest stages of brain development. References: Ek et al. (2006). J Comp Neurol 496:13-26.

POS-MON-024

CEREBROSPINAL FLUID SECRETION DURING EARLY BRAIN DEVELOPMENT

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In the adult cerebrospinal fluid (CSF) is produced by the actions of many transporters and enzymes which create ion gradients that drive the entry of water into the ventricles (mainly via aquaporin-1 water channels). It is not known when in development CSF secretion starts but, in the rat, it has been postulated to occur around birth. However, recent evidence suggests that the secretion starts much earlier, even as soon as the choroid plexuses appear (Johansson et al., 2006). **Purpose:** To investigate the developmental profile of the two major enzymes responsible for CSF secretion in the adult, Na,K-ATPase and carbonic anhydrase II. **Methods:** The developmental profiles of both enzymes were investigated using immunohistochemistry and Western Blot analysis of choroid plexuses from embryonic day (E) 15, 18, postnatal day (P) 0, P9 and adult rats (n=3 for all ages). **Results:** Western Blot analysis showed a progressive increase in the amount of Na,K-ATPase relative to total protein with age, with very low levels at E15. Immunohistochemistry confirmed the presence of Na,K-ATPase in the lateral ventricular choroid plexus from E15 onwards. Carbonic anhydrase II seems to appear in the lateral ventricular choroid plexus between P0 and P9. **Conclusions:** The low levels of Na,K-ATPase and the absence of carbonic anhydrase II during early choroid plexus development indicate that other mechanisms may be involved in CSF secretion, a process that is crucial for normal brain development. Johansson et al., 2006. Eur J Neurosci. Vol 24 pp. 65-76.

POS-MON-025

DEVELOPMENT OF CORTICAL FEEDBACK CONNECTIONS TO FERRET STRIATE CORTEX

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Purpose: Visual cortical areas in the mammalian adult brain are interconnected by a complex network of interareal feedforward and feedback circuits. We investigated the postnatal development of feedback connections to ferret primary visual cortex. Our aim was to determine whether feedforward and feedback cortical circuits follow similar developmental timecourses. **Methods:** We injected the neuronal tracer cholera toxin B subunit (CTb) into primary visual cortex of juvenile ferrets (n=9) to visualize the distribution and pattern of retrogradely labeled cells in extrastriate cortex. **Results:** As in the adult, up to postnatal day 42 we observed extensive label spreading within area 17, and a large number of retrogradely labeled cells in areas 18, 19, 21 and the suprasylvian cortex. Unlike the adult, we also found retrograde label in inappropriate areas such as primary auditory and posterior ectosylvian cortex, and substantial retrograde label in lateral temporal visual areas. By postnatal day 42, retrogradely labeled cells and orthogradely labeled terminals formed discrete overlapped clusters in each extrastriate area, indicating reciprocal feedforward and feedback connections with area 17. Between postnatal days 42-55, an essentially adultlike pattern of connections emerged, with a loss of inappropriate connections, and a reduction in the number and spatial extent of labeled cells in each extrastriate area. **Conclusion:** Cortical feedback projections to ferret primary visual cortex appear to refine to their adultlike state during the second postnatal month, a period in which the eyes are open. Visual experience is likely to play a critical role in this refinement process.

POS-MON-026

DO WE SELECTIVELY ADAPT TO SURFACE REFLECTANCE?

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Purpose: 'Colour constancy' refers to our ability to judge the reflectance of surfaces under changing illumination. While most models of this process assume a neural representation of surface reflectance, there is little direct evidence for this. We used psychophysical adaptation to test for the presence of such a neural population. **Methods:** Stimuli were Mondrian-like patterns of overlapping surfaces surrounding a central surface, presented on a computer monitor. To induce adaptation, stimuli with a common central surface (eg. red) were viewed for one minute. Throughout the adapting period, each surface was rendered under each of six illuminants, which updated at a rate of 4 Hz. In Condition 1, all surfaces were rendered under the same illuminant, consistent with a constant scene under changing illumination. In Condition 2, each surface was separately rendered under a randomly chosen illuminant, simulating instead a changing scene. Over time, adapting stimuli had identical distributions of reflected wavelengths. During adaptation subjects classified the colour of a test surface, which briefly replaced the central adapting surface, as red or green. We expect that adaptable neural mechanisms representing surface reflectance will be desensitised most in Condition 1, where the adapting display is consistent with an unchanging central surface. **Results:** Classification boundaries shifted towards the adapting surface in both conditions. In 3 of the 6 subjects, this shift was significantly greater ($p<0.05$, sign test) in Condition 1 than Condition 2. In the others there was no significant difference. **Conclusion:** The greater shifts seen in Condition 1 are evidence for the presence of adaptable mechanisms that are sensitive to surface reflectance.

POS-MON-027

ACCURATE ESTIMATION OF SELF-MOTION BY IDENTIFIED NEURONS IN THE HOVERFLY VISUAL SYSTEM

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Purpose: Despite limited resolution compound eyes and tiny brains (<1 million neurons) flies are able to engage in exquisitely controlled aero-navigational feats. In the fly visual system an extensively studied class of neurons, the horizontal system cells (HS), are proposed to be involved in the detection of self-motion. We aimed to investigate the performance of HS neurons as estimators of self-motion when presented with a range of naturalistic stimuli. **Methods:** Sharp electrode, intracellular recordings were performed on unanaesthetised hoverflies, *Eristalis tenax* (n=92), whilst presenting visual stimuli on a high refresh rate CRT. 13, 360 degree, outdoor panoramic images were collected and displayed to simulate naturalistic yaw rotation. **Results:** Detailed receptive field information was obtained for 55 different HS neurons and we went on to test the responses to all 13 panoramic images on 6 occasions. Amazingly, when presented with natural scenes, the HS neurons coded velocity almost identically despite large variations in contrast and spatial structure from one image to the next. **Conclusion:** HS neurons in the hoverfly visual system are able to respond in a fashion that is consistent with them providing accurate estimations of yaw rotation to higher-order centres of the brain even when presented with a diverse range of natural scenes. Despite a wealth of knowledge on motion detection in the insect visual system current models are unable replicate the robust velocity responses observed.

POS-MON-028

CONNECTIONS OF THE PRIMATE FRONTAL POLE

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High-order cognitive functions depend not only on interconnections between prefrontal cortical areas, but also on long-range interconnections with sensory association areas. Little is known about the neural pathways to area 10, located at the frontal pole and believed to play a role in directing attention to unexpected events and the planning of future actions. We investigated the extent of these connections by placing four retrograde tracers in different parts of this region in three anaesthetised marmoset monkeys (Alfaxan, 4 mg/kg initial dose). The predominant input to polar area 10 originates bilaterally from other prefrontal areas, the contralateral component comprising approximately one third of the total. The strongest contralateral input originates from homologous parts of area 10. A sparse ipsilateral projection from premotor areas predominantly targets lateral area 10. Ipsilateral sensory projections comprise approximately 15-60% of the total input, the strongest component (visual) originating from the inferior temporal area. Sensory projection neurons are also located in the fundus of superior temporal area (FST) and in the putative superior temporal polysensory area (STP). Smaller inputs originate in parahippocampal and retrosplenial visual association areas (TF, TH and prostriata). Medial injections also labelled neurons in auditory association area (parabelt). Surprisingly, there is no input from parietal or somatosensory cortices. We can conclude that cognitive processing involving area 10 is strongly bilateral, and chiefly dependent on other prefrontal regions. Direct somatosensory information is not utilised, but inputs from caudal areas suggest that area 10 has access to visual and auditory information about object recognition as well as spatial information.

POS-MON-029

ORIENTATION SELECTIVITY OF SINGLE NEURONS IN CAT STRIATE CORTEX: MEAN FIRING RATES VS. PHASE-VARIANT COMPONENT OF SPIKE RESPONSES

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Virtually all neurons in the mammalian primary visual cortices can be identified quantitatively as simple or complex on the basis of the ratio of the phase-variant (F1) component to the mean firing rate (F0) of their spike responses to luminance-modulated sine-wave gratings drifting through their classical receptive fields (CRFs). While both simple (strongly phase-variant; F1/F0 >1) and complex (weakly phase-variant; F1/F0 <1) cells are orientation-selective, the mechanism(s) underlying orientation selectivity are hotly debated. **Purpose**: To determine if the mean firing rate (F0) and F1 components of responses of neurons in mammalian primary visual cortex are tuned to the same orientations. **Methods**: We recorded spike responses of single neurons from the striate cortices of anaesthetized, paralysed and artificially ventilated adult cats (16). **Results**: There were 180 cells in which F0 and/or F1 component of spike responses to optimised (spatial and temporal frequencies, orientation) high-contrast gratings drifting across their CRF were at least 15 spikes/s. In all simple cells but one (94/95) and a substantial majority (57/85;68%) of complex cells, both F1 and F0 were tuned to virtually the same (±10°) orientations. However, in over 15% (15/85) of complex cells, only F0 was orientation tuned while in ~15% (12/85), F0 and F1 were tuned to orientations differing by 20-120°. **Conclusions**: Our data suggest that: 1) the mechanism underlying the orientation selectivity of complex cells in cat striate cortex might be indeed heterogeneous and 2) some complex cells (F0 and F1 tuned to different orientations) might provide useful cues concerning the texture of visual stimuli.

POS-MON-030

SOURCE AND CHARACTERISTICS OF CORRELATED ACTIVITY IN THE MARMOSET'S LATERAL GENICULATE NUCLEUS

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Purpose: Correlated activity among cortical neurones may underlie high-order processes such as intra- and cross-modal stimulus binding. Here we asked whether correlated activity is also present in the cortical afferent stream (dorsal lateral geniculate nucleus, LGN). **Methods:** Extracellular recordings from pairs of neurones were made from the LGN of sufentanil-anaesthetised marmosets (n = 4) using a single electrode and software spike discrimination. Receptive field centre sensitivity, size and overlap were calculated from responses to drifting gratings. Responses in the absence of spatial contrast ("spontaneous activity") were also measured. Correlation was estimated from Z-transformed average firing rates and cross-correlations across 2-3 second trials spread over several minutes. **Results:** Spontaneous and evoked activity of like centre-polarity (ON/ON and OFF/OFF) neurone pairs was positively correlated (mean Z-transformed correlation for evoked activity: 0.38, range 0.10 to 0.88, n = 5), whereas spontaneous and evoked activity of opposite centre-polarity (ON/OFF and OFF/ON) neurone pairs showed weaker and negative correlation (mean = -0.06, range -0.12 to 0.00, n = 4). This indicates that the correlations most likely originate in the retina and are preserved in the LGN. Correlations were present in parvocellular (n = 7) and magnocellular (n = 2) cell pairs, and in overlapping and non-overlapping receptive field pairs. Cross-correlation analysis showed these effects occur over long (multi-second) time-scales, consistent with contribution of modulatory inputs to correlation. **Conclusions:** Stimulus-independent response correlations are a consistent feature of responses in LGN neurones. The effect of these correlations would be to reinforce activity of members of the ON and OFF pathways at spatial scales larger than served by individual receptive fields.

POS-MON-031

LATERAL GENICULATE NUCLEUS IN MARMOSETS: A COMPARISON OF DICHROMATIC AND TRICHROMATIC ANIMALS

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Purpose: The parvocellular (PC) division of the dorsal lateral geniculate nucleus (LGN) is considered to carry signals for red-green colour vision in trichromatic primates. We asked whether the PC layers in trichromatic and dichromatic ("red-green colour blind") marmosets show differences in cell number and/or retinal innervation. **Methods:** Retinal afferent terminals in the PC layers were labelled by iontophoretic injections of rhodamine-conjugated dextran and/or biotinylated dextran amines in sufentanil-anaesthetised marmosets (n = 15), and reconstructed from series of 50 µm coronal sections. Nissl-stained cell density and volume of all the LGN layers from these and other marmosets (total n = 24) were also calculated. **Results:** A total of 41 LGNs (25 dichromats, 16 trichromats) was analysed. There was a strong correlation (r = 0.91) in volume of the left and right LGNs taken from the same animal, but negligible correlation of LGN volume with body weight, age, or gender. No difference was seen on comparing the average volume of PC layers (dichromats: mean = 3.6, SD=0.4 mm^3, n = 25; trichromats mean = 3.3, SD=0.5, n = 16, p = 0.11, Wilcoxon non-parametric rank-sum test), the density of Nissl-stained neurones (134 x 10^3, SD 51 x 10^3, n =30 vs. 138 x 10^3, SD 45 x 10^3 cells / mm^3, n = 35, p = 0.6), or retinal afferent arbour volume (0.15 x 10^{-3}, SD 0.12 x 10^{-3}, n = 30 vs. 0.12 x 10^{-3}, SD 0.07 x 10^{-3}, p = 0.7) **Conclusions:** The anatomical organization of the PC layers is similar in dichromatic and trichromatic marmosets.

POS-MON-032

EFFECT OF DOPAMINE ON RETINAL GANGLION CELLS

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Purpose: Retinal ganglion cells provide the output from the retina for both rod-mediated responses in low light conditions (scotopic), and cone-mediated responses during higher light levels (photopic). Dopamine release in the retina is stimulated by increasing ambient light intensity, and acts throughout the retina by volume transmission. Dopamine is thought to block rod-mediated light responses to facilitate cone-mediated transmission through ganglion cells to higher visual processing areas. Thus the aim of this study was to examine the effect of dopamine on signal transmission and on the receptive field properties of ganglion cells in low light conditions. **Methods:** We performed patch-clamp recordings on ganglion cells in isolated mouse retinal whole-mounts in scotopic conditions. Light stimuli designed to characterise receptive field properties such as centre/surround organisation and spatial and temporal frequency sensitivity were displayed and responses were recorded in current- and voltage-clamp modes. Dopamine was then introduced via the extracellular solution, and procedures were repeated. **Results:** Dopamine reduced the strength of light responses in ON ganglion cells (n=5). Area-response functions of ON cells under dopamine were shifted towards larger diameters. Responses to gratings modulated for spatial and temporal frequencies were also significantly reduced. Dopamine had no effect on responses of light responses in ON/OFF ganglion cells (n=4). **Conclusion:** The reduction of the responses in ON cells is consistent with the role of dopamine as a switch between rod and cone circuits. The significance of the lack of dopamine effect on ON/OFF cells is unclear at this stage. A series of experiments exploring the effects of dopamine on excitatory and inhibitory conductance is currently being pursued to elucidate its mechanisms of action.

POS-MON-033

ON DIRECTION-SELECTIVE GANGLION CELLS: SYNAPTIC MECHANISMS UNDERLYING TEMPORAL DYNAMICS

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Purpose: There are two types of direction-selective ganglion cells (DSGCs) in the retina: the well characterised On-Off DSGCs and the less common On DSGCs. This study compared the synaptic inputs that shape the receptive-field properties of the two types in the rabbit retina. **Methods:** DSGCs were microscopically targeted based on their somatic appearance in the isolated superfused retina and the receptive-field properties were mapped by extracellular spike recordings. The cell was then voltage-clamped, the light-evoked synaptic currents recorded over a range of holding potentials, and conductance analysis used to estimate the relative excitatory and inhibitory synaptic inputs. **Results & Conclusions:**The synaptic mechanisms underlying the generation of direction selectivity appear to be similar in the two cell types. Like the On-Off DSGCs (Taylor & Vaney, 2002), the On DSGCs receive both directional excitatory inputs and directional inhibitory inputs (n=13). Furthermore, somatic application of tetrodotoxin unmasked presumed dendritic spikes in On DSGCs (n=4), similar to those found in On-Off DSGCs (Oesch et al. 2006). However, the synaptic mechanisms underlying the temporal dynamics of the two types differed in several important respects. Moving grating and flicker stimuli revealed that the On DSGCs (n=5) receive a qualitatively different pattern of excitation and inhibition from the On-Off DSGCs (n=5). Our results suggest that the transient bipolar cell providing the excitatory input to the On-Off DSGCs may drive an amacrine cell that inhibits the On DSGCs, whereas the sustained bipolar cell providing the excitatory input to the On DSGC may drive an amacrine cell that inhibits the On-Off DSGCs.

POS-MON-034

POSTNATAL A2A ADENOSINE RECEPTOR LOCALISATION IN THE DEVELOPING RAT RETINA

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Adenosine has been implicated in the regulation of neuronal apoptosis through interactions at the A2a adenosine receptor (A2aR). **Purpose:** In order to further understand the role of the A2aR in the developing retina, we aimed to localise to A2aR during postnatal development. **Methods:** Retinas from postnatal day (P)0, P2, P4, P7, P11 and adult rats (n ≥ 3 at each age) were lightly fixed with 4 % paraformaldehyde and cryostat sections were immunolabelled. **Results:** A2aR-like immunoreactive (A2aR-IR) puncta were detected in the ganglion cell layer from P0. A subset of these neurons were Brn-IR suggesting that at least some of these neurons are ganglion cells. From P2 onwards, A2aR-IR puncta appeared in the developing inner nuclear layer in a subset of amacrine-like neurons. By P4, a subset of neurons contain A2aR-IR puncta were detected in the regions expected to be horizontal cell somata. These neurons were also transiently ChAT-IR. ChAT-IR neurons in the inner third of the inner nuclear layer and ganglion cell layer were also A2aR-IR, suggesting starburst amacrine cells express this receptor. P7 and older ages showed less A2a-IR in the ganglion cell layer and the inner third of the inner nuclear layer while A2a-IR in horizontal-like cells persisted into adulthood when these neurons were calbindin-28_IR. **Conclusion:** A2a adenosine receptor localisation changes during the postnatal development of the retina suggesting adenosine may have a role in the postnatal development of the rat retina.

POS-MON-035

GROUP III METABOTROPIC GLUTAMATE RECEPTORS IN PRIMATE RETINA: AN IMMUNOCYTOCHEMICAL STUDY

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Purpose: To analyse the distribution of group III metabotropic glutamate receptors (mGluRs) in marmoset retina. **Methods:** Antibodies against the group III metabotropic glutamate receptors, mGluR4, mGluR7, and mGluR8 were applied to vertical cryostat sections of lightly fixed marmoset retina. Excitatory synapses were identified with antibodies to the C-terminal binding protein CtBP2 (presynaptic bipolar ribbons) or antibodies to PSD-95 (postsynaptic density protein 95). Five bipolar types were identified with various immunohistochemical markers as described previously (Chan et al., 2001, JCN). **Results:** Punctate immunofluorescence was observed throughout the inner plexiform layer for all group III mGluRs but was less dense close to the ganglion cell layer. Double-immunofluorescence revealed that all group III mGluRs studied are more strongly associated with the postsynaptic marker PSD-95 than with the marker for presynaptic ribbons (CtBP2). Flat midget bipolar cells, and the diffuse bipolar types DB3 and DB4 had comparable numbers (3 to 5) colocalised mGluR immunoreactive puncta per axon terminal. Rod bipolar and DB6 cells showed no significant association with group III mGluRs. **Conclusions:** The results suggest that mGluR4, 7 and 8 have a postsynaptic location in the retina.

POS-MON-036

MICROARRAY ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN THE DEVELOPING FOVEA

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Purpose: In humans, retinal vessels form at the optic disc at 14-15 weeks gestation (wg) and follow a stereotypical pattern of development that includes reduced vascular density in the macula and definition of a foveal avascular zone. The aim of this investigation is to identify genes expressed in the macula which may be involved in patterning and definition of the foveal avascular zone. **Methods:** Biopsies were taken of the foveal ('fovea') and nasal ('nasal') regions of 3x19 wg and 1x20 wg foetal eyes. High integrity RNA was extracted from each biopsy and from the remaining retina ('surround'). Three RNA samples from each specimen (12 in total) were applied to an Affymetrix HG-U133 Plus 2.0 GeneChip® microarray, hybridized, then scanned. Differential expression between the 'fovea' and non-fovea arrays was determined using Partek, followed by investigation of gene ontology (DAVID; GeneSpring). **Results:** More than 4000 genes are differentially expressed between the fovea and non-foveal arrays ($p < 0.01$). About half are linked with biological processes that distinguish the more mature foveal region with the poorly differentiated peripheral retina (eg. cell cycle, metabolism and phototransduction) and have been discounted. Genes associated with axon guidance are highly represented in the remaining pool. **Conclusion:** Primate retinal vessels follow a pattern similar to that established by ganglion cell axons, and axonal and vascular guidance mechanisms share many genes in common. We suggest, therefore, that the group of genes identified in this study have important roles in regulating retinal vascular development and patterning.

POS-MON-037

GLIAL CELL ABNORMALITIES OCCUR CONCURRENTLY WITH NEURAL DYSFUNCTION AND HYPOXIA IN THE RETINA DURING DIABETES

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Purpose: Diabetic retinopathy is the leading cause of blindness in working-aged adults. In addition to vascular abnormalities, there are alterations in neuronal and glial function. This project attempted to characterize the time course of neuronal, glial changes in relation to the development of hypoxia during diabetes. **Materials and Methods:** Sprague-Dawley rats were rendered diabetic by an i.v injection of streptozotocin (50mg/kg). Control rats received injections of citrate buffer alone. Following 1, 4, 6 and 12 weeks of diabetes (N=10-14 per timepoint), retinal function was assessed using the electroretinogram. Retinal hypoxia was assessed using the Hypoxyprobe system; pimonidazole was injected intravitreally. Following 3 hours retinae were fixed in 4% PF and processed for pimonidazole immunocytochemistry. Changes in retinal glia were assessed using immunocytochemistry with GFAP, S100b, Connexin-26, EAAT4 and P2Y1. **Results:** Rod photoreceptor and cone post-receptoral losses, were detected following 6 weeks of diabetes. Hypoxyprobe labeling was detected in the Inner Plexiform Layer, ganglion cells and Müller cells at 6 weeks. Hypoxyprobe labelling of Müller cells colocalised with upregulated GFAP in their processes. Evaluation of gliotic changes using Connexin 26 immunolabelling revealed the protein was decreased in peripheral diabetic retinae. In flatmounted diabetic retina, Connexin 26 indicated a change in astrocyte morphology. **Conclusions:** These data suggest glial cell anomalies occur concurrently to the development of hypoxia and neuronal dysfunction in the retina during diabetes. Further work will be necessary to determine the precise relationship between glia and the retinal vasculature during diabetes.

POS-MON-038

DEVELOPMENT OF FUNCTIONAL CIRCUITS WITHIN THE RETINA PRECEDES VASCULAR DEVELOPMENT IN THE RETINA

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Aim: Vascular development within the retina is known to depend on expression of the angiogenic growth factor, VEGF in response to tissue hypoxia. What is not clear is whether an increase in neural activity within the retina induces physiological hypoxia that precedes the formation of the intra-retinal vasculature. The aim of this study was to examine the development of retinal circuits in relation to the formation of the superficial and deep vascular plexus. **Methods**: Sprague-Dawley rat retinae from birth to adult were either processed for indirect immunofluorescence immunocytochemistry or incubated for 5 minutes in a physiological saline solution containing agmatine (25mM), to probe for neural function (n=3 per age). Following fixation in 4% paraformaldehyde, the tissue was sectioned and processed for immunocytochemistry. Antisera known to identify specific cell types, synaptic proteins, and vascular endothelial cells were used. **Results**: Functional activation of amacrine cells and ganglion cells was observed by P1, before the development of the superficial vascular plexus. This was followed by development of the cone pathways, from the distal to proximal retina. Cone and very few rod terminals were present by P2-P6, followed by development of OFF cone bipolar cells and finally rod bipolar cells. Functional activation of cones to horizontal cells was evident from P6. The development of the cone and rod pathway occurred before the development of the deep vascular plexus at P9. **Conclusion**: These data suggest that development of the vasculature occurs after the onset of neural activity and maturation of retinal circuits. Further work is necessary to determine whether maturation of the cone pathways in particular initiates physiological hypoxia that drives vascular growth within the distal retina.

POS-MON-039

TEN_M3 IS REQUIRED FOR THE DEVELOPMENT OF TOPOGRAPHY IN THE IPSILATERAL RETINOCOLLICULAR PATHWAY

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Purpose: Retinal projections are organised into topographic maps within their two main targets, the dorsal lateral geniculate nucleus and the superior colliculus (SC). Little is known about the mechanisms which regulate the development of topographic projections from the ipsilateral eye. Recent work from our lab has shown that the transmembrane glycoprotein plays a key role in the development of ipsilateral retinogeniculate projections. The current study had three aims: 1. Examine the normal development of the ipsilateral retinocollicular pathway. 2. Determine whether ten_m3 is required for the generation of topography in the SC. 3. Examine the expression of other guidance molecules implicated in retinocollicular mapping such as the EphrinAs and EphAs in ten_m3 knockout (KO) mice. **Methods**: Injections of anterograde fluorescent tracers in developing wildtype and ten_m3 KO mice were used achieve aims 1 and 2. Alkaline phosphatase bound protein probes were used to achieve aim 3. **Results**: Ipsilateral axons are largely targeted to the appropriate mediolateral region of the SC from their earliest ingrowth but overshoot their termination zones in the rostrocaudal axis (n=3). Topography develops by the extension of interstitial branches at topographically appropriate regions. Ten_m3 KOs have significant changes in the organisation of ipsilateral retinocollicular projections as assessed by both bulk-fills (n=5) and focal injections (n=4). Moreover, EphA expression was significantly down regulated in the SC (n=2). **Conclusion**: These findings provide evidence for topographic order in the development of the ipsilateral projection from early stages of development. The work also demonstrates that ten_m3 plays an important role in the development of ipsilateral projections to the midbrain and provides a potential mechanistic explanation for the observed changes in topography.

POS-MON-040

THE ABSENCE OF TEN_M3 LEADS TO AN INTEROCULAR MISMATCH IN PRIMARY VISUAL CORTEX

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Purpose: Previous work from our lab has shown that mice lacking Ten_m3 exhibit an altered distribution of ipsilateral retinal projections. In Ten_m3 knockout (KO) mice, ipsilateral terminations are present in the region of the dorsal lateral geniculate nucleus that projects to the medial, normally monocular, portion of the primary visual cortex (V1). The representation of ipsilateral information in the normally monocular region of V1 is a potential cause of deficits in visual behaviour we have reported. **Methods:** Transneuronal labeling, immunoreactivity for c-fos and single unit electrophysiological recordings were used to assess the presence of ipsilaterally driven cells in medial V1. **Results:** Transneuronal labeling in KOs (n=3) confirmed the presence of ipsilateral inputs to V1 in Ten_m3 KO mice which were not present in WTs (n=2). Expression of c-fos following binocular stimulation was markedly lower in KOs versus WTs. In monocularly inactivated KOs (n=6) patches of high expression were seen in medial V1. Single unit *in vivo* electrophysiological recordings from medial V1 showed the presence of dual receptive fields which differed in location for each eye in KOs; in contrast, single receptive fields were seen in WTs. **Conclusions:** The altered ipsilateral retinogeniculate projection observed in the absence of Ten_m3, leads to the presence of ipsilaterally driven cells in normally monocular V1. This aberrant ipsilateral drive causes an interocular mismatch which may lead to suppression in V1. This may be responsible for the observed behavioural deficits in Ten_m3 KO mice.

POS-MON-041

MEDIUM DRG NEURONS EXPRESSING TRPV1, NOS, CGRP AND SP INNERVATE THE ADULT MOUSE JEJUNUM

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Purpose: Pain originating from the gastrointestinal tract is thought to be mediated by spinal afferents arising from the dorsal root ganglia (DRG). Based on their conduction velocities, these visceral afferents are almost exclusively *small* diameter myelinated Aδ- or unmyelinated C-fibres. However, quantitative information about sizes and chemical phenotypes of DRG neurons supplying the small intestine is poor. We determined the size distribution and expression patterns of histochemical markers - transient receptor potential vanilloid 1 (TRPV1), calcitonin gene-related peptide (CGRP), substance P (SP), neuronal nitric oxide synthase (NOS) and isolectin B_4-binding (IB_4) - in DRG neurons supplying the jejunum. **Methods:** C57Bl6 mice (n=14) were anaesthetized and Cholera toxin B (CTB; 0.1 µL/injection) was injected into the jejunal wall. Leakage of dye was prevented with a thin layer of cyanoacrylate. Animals were sacrificed 7-10 days after treatment and DRGs (T8-T13) removed, cryosectioned and processed for multi-label immunofluorescence of different markers. Soma size was analysed using ImageJ software (NIH). Size distributions of each class of neurons were compared using Kolmogorov-Smirnov tests. **Results:** CTB-labelled jejunal afferent neurons were predominantly *medium-sized* (300-600 µm^2) and *large-sized* (> 600 µm^2) cells. This was not due to preference of CTB for larger cells since both CTB and an alternative tracer, Fast blue, labelled similar cutaneous afferent populations including small-sized (< 300 µm^2) neurons. CTB-labelled DRG neurons expressed TRPV1, CGRP, SP or NOS, but lacked IB_4-binding. NOS and SP were almost always co-localized with TRPV1 and CGRP. **Conclusion:** More than half of the spinal afferents innervating the jejunum are medium sensory neurons that express TRPV1, CGRP, SP and NOS.

POS-MON-042

EXPRESSION OF SEROTONIN SYNTHESISING ENZYMES IN MOUSE TRIGEMINAL GANGLIA

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Purpose: Serotonin (5-HT) and its receptors have been proposed to play a key role in the pathogenesis of migraine. A recent study (Headache, 2006 Sep; 46(8):1230-45) proposed that the trigeminal ganglion (TG) is a source of 5-HT in female mice. This study found tryptophan hydroxylase (TPH) was expressed in TG and also that a significant proportion of TG neurons contained 5-HT. Migraine is a disorder with a markedly higher occurrence in females compared with males, so in the present study we compared the expression of the mRNA for each of the enzymes involved in the 5-HT synthesis and transport between male and female mice. **Methods:** Trigeminal ganglia from 6-8 week old C57 BL/6 mice (n=8 for each sex) were dissected and RNA isolated using TRI reagent. Primers were designed for TPH isoforms 1 and 2, amino acid decarboxylase (AADC), the 5-HT transporter (SERT) and the house keeping gene, 3-phosphoglycerate kinase (PGK). Following PCR, the amount of mRNA for each test gene was compared to PGK using densitometry and normalized to PGK expression in each sample. **Results:** In this study, TPH1, TPH2, AADC and SERT mRNA were present in male and female mice. The level of TPH1 and TPH2 expression was greater in males (1.14±0.14, 0.34±0.03) than in females (0.73±0.06, 0.23±0.01, P<0.05). The level of SERT and AADC expression did not significantly differ between males and females. **Conclusions:** These results indicate that all the enzymes required for serotonin synthesis and its transport are present in both male and female mouse TG, and TPH1 and TPH2 appear to be expressed at higher levels in male mice.

POS-MON-043

CENTRE-SURROUND PROCESSING IN THE OLFACTORY BULB IS TEMPORAL, AND INTRINSIC TO THE LEVEL OF SENSORY INPUT

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Purpose: Odours are encoded in the olfactory bulb (OB) as spatial patterns of activity. Centre-surround inhibition between different regions of the OB may increase contrast in these spatial patterns. This may occur at two separate levels of circuitry within the OB; when OB output neurons (mitral cells) interact with inhibitory granule cells in the external plexiform layer (EPL), at the level of OB output to higher brain centres and between sites of olfactory nerve (ON) input to mitral cells, in the OB glomerular layer (GL). Through this circuit, ON excitation of one odorant-specific GL region might inhibit mitral cell responses in surrounding regions that have different odorant specificity. We have investigated the effect of the timing of ON input to the OB on centre-surround inhibition within the OB. **Methods:** We investigated this circuit in mouse OB slices *in vitro* with single-unit extracellular recording and whole-cell recording of mitral cells. **Results:** Synaptic ON input to one region of the GL can inhibit responses to ON input in other regions, but only after ~200ms, increasing in effectiveness with increasing time intervals (one-way ANOVA n=10, p=9.28E-5). **Conclusion:** Centre-surround inhibition *in vitro* occurs *exclusively* at the stage of synaptic input to (GL), rather than output from the OB (EPL). We suggest that the latter form of processing requires feedback from other brain regions and the delay in inhibition corresponds to the time course of mutual excitation between odorant-specific groups of mitral cells, which amplifies excitatory responses to ON input and resists inhibition from other regions with different odorant specificity. Odour discrimination is thus enhanced at the earliest stage of olfactory processing.

POS-MON-044

PROFESSIONAL SNIFFER'S: ABILITY, USE AND PERCEIVED IMPORTANCE OF SMELL

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Purpose: The ability to smell is an important component in the arsenal of healthcare professionals diagnostic tool kit. Breath, tissue and other odours often contribute to clinical diagnosis and the instigation of treatment regimens. Despite this, we have yet to find a published study that examines smelling ability in a professional group or students training in a specific profession, irrespective of how significant smelling is in that profession. Thus we explored the sense of smell in experienced nurses and student nurses in comparison to the normative Australian population. **Methods:** This study applied the sniffin' sticks test of olfactory ability, coupled with simple questionnaire data, to a group of nursing students (n=20) and experienced nurses (n=20). These data were compared to demographically similar Australian data in non-health professionals using the current Australian olfactory data base held at GU. **Results:** Nurses reported much greater attention to and importance of their sense of smell than either of the other groups. However, there were no significant differences (p>0.05) between the actual or perceived ability to smell by nursing students, nurses and the normative Australian population for odour identification, odour discrimination or threshold for odour detection. **Conclusion:** This study is a significant beginning to our understanding of the use and importance of olfaction in the work-place. It enables us to begin to build an understanding of how attention to olfactory cues (often indicating disease) may develop during clinical experience and thus to understand how we can best prepare students in healthcare and medical associated professions for their future 'information-rich' clinical olfactory environment.

POS-MON-045

GENETIC ASSOCIATION BETWEEN VARIATION OF THE TATA BOX-BINDING PROTEIN AND HUMAN LONGEVITY

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Spinocerebellar ataxia type 17 (SCA17) is a rare polyglutamine disease caused by expansion in the polyglutamine coding tract within the TATA box-binding protein (TBP) gene. While 43 or more repeats are required to cause SCA17, wild-type TBP alleles most commonly carry 37 or 38 repeats. Our research group has published studies providing pathological and functional evidence that suggests wild-type TBP may also play a role in neurodegeneration. **Purpose**: We set out to test the hypothesis that the polymorphic polyglutamine tract within wild-type TBP may be part of a general neurodegenerative mechanism and thereby contribute to some of the variability in human longevity. **Methods**: TBP haplotypes were generated for 743 long-lived individuals (aged 95 to 110 years) and 511 control individuals (aged 60 to 75 years). **Results**: Polyglutamine coding polymorphism in the TATA box-binding protein was found to be associated with life expectancy (P 0.0003). Genetic association results will be presented and discussed. **Conclusion**: We propose that this association between TBP variation and longevity is likely due to a contribution of the polyglutamine repeat to common neurodegenerative disorders.

POS-MON-045A

SPATIAL VARIATIONS IN MOTOR UNIT FORCES OF THE FDI

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Purpose: Earlier studies in multifunctional muscles show that the control of motor units (MUs) can vary as a function of force direction. While directionally dependent motor unit recruitment implies that there may also be differential mechanical action, this has yet to be demonstrated. Our objectives were to determine whether there exists a range of force vectors from different motor units in the FDI muscle. **Methods**: In 15 neurologically intact subjects, the index finger was attached to a six degrees-of-freedom load cell. To record MU activity, fine wire electrodes were inserted into the FDI. We utilized the spike-triggered averaging (STA) method to derive force twitch vector estimates from single motor units. We derived MU twitch direction from the ratio of individual twitch estimates recorded concurrently from the load cell. **Results**: Eighty four units from 15 subjects were collected. We were able to estimate force twitch vectors from 3 to 10 different MUs in each of 15 subjects. The range of MU vectors within individual subjects varied from a minimum of 26 degrees to a maximum of 90 degrees with an average value of 53 degrees. These estimates were significantly different ($p<.01$) from the average variability of 13 degrees from repeat estimates of the same motor unit. Pooled data of all MUs recorded from all subjects shows that the MU force vector angles vary from a minimum of 137 degrees to a maximum of 270 degrees. **Conclusion**: The results suggest that there is varied mechanical action of motor units in the FDI. It is possible that varied mechanical action of the whole muscle is based on differential activation of individual MUs in the FDI.

POS-MON-046

ULTRASTRUCTURAL ANALYSIS OF HEREDITARY SENSORY NEUROPATHY TYPE 1 (HSN1) PATIENT LYMPHOBLASTS

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Functional abnormalities which prevent mitochondria travelling to the distal end of the nerve have been proposed as a possible cause of length-dependent axonal degeneration. Hereditary sensory neuropathy type 1 (HSN1) is a typical length-dependent axonal degenerative disorder. The mutation is in the gene for serine palmitoyltransferase (SPT) long chain subunit 1 (SPTLC1). SPT is an integral endoplasmic reticulum (ER) membrane protein, and it is unclear how such mutations might affect mitochondrial function. We have examined mitochondrial function in lymphoblast cells (transformed lymphocytes) from patients with two different mutations in SPTLC1, Cys133Trp and Val144Asp, and compared them to cells derived from healthy volunteers to better understand the potential basis of the neurodegeneration. Transmission electron microscopy on lymphoblast cells from patients with HSN1 revealed several mitochondrial structural abnormalities. Mitochondria were swollen and their cristae were abnormal. The cristae were unusually shaped, shortened and were decreased in abundance. Many mitochondria exhibited small discontinuities in the integrity of their outer membrane, suggestive of functional damage. They were also clustered in a peri-nuclear region rather than being well dispersed in the cytoplasm. A few mitochondria were enveloped in rough ER. The amount and distribution of ER in cells from patients was otherwise not affected, but the ER was swollen. Surprisingly, the cellular levels of ATP were unaltered in patient cells. We propose that the mitochondria in cells from HSN1 patients are functionally compromised. The results suggest a surprising connection between a resident ER protein and mitochondrial integrity that may play an important role in the distal axonal degeneration characteristic of this disease.

POS-MON-047

HFI-1 DECREASES RECOVERY TIME AND REDUCES NEUROLOGICAL DAMAGE IN A PARTIALLY REVERSIBLE SCI MODEL IN RATS

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Spinal cord injury (SCI) predominantly occurs in young adults leading to permanent paraplegia and quadriplegia, and increased costs to society. Our research focuses on developing drugs to minimise the effects of SCI. Thus we undertook a study of a mexiletine analog, HFI-1, which has a sodium channel blocking pharmacophore linked to an antioxidant moiety, to assess it as a neuroprotectant. Male Hooded Wistar rats were anaesthetised (2% isoflurane/98% oxygen), and laminectomy performed at spinal level T12. An inflatable balloon catheter was inserted rostral, underneath the vertebra, to T10 and inflated for 5 minutes, causing reversible paraplegia. This model demonstrates a slow, graded return of hindlimb motor function over 15 days, which allows assessment of putative neuroprotective drugs to accelerate recovery. Rats had almost complete functional recovery by 15d. Mexiletine (12.5mg/kg, i.p.; n=8), HFI-1 (6mg/kg; 30mg/kg, i.p.; n=10&9) or vehicle (n=11) were administered at 3h after the injury and twice daily thereafter, until killed. Behavioural tests were conducted every 3d. At 15d post-injury, rats were anaesthetised and transcardially perfused, to fix the spinal cords. Sections were cut and processed to examine the size of the cyst and modulatory effects of HFI-1 on lesion formation. HFI-1 treatment significantly decreased recovery time in behavioural outcomes following SCI, as seen in the ladder walking test, BBB scale and inclined ledged beam; with differences from untreated rats at 9d and 12d ($P<0.05$). Vehicle-treated rats only showed significantly improved behavioural recovery at 12d and 15d post-SCI. Mexiletine and HFI-1 reduced volume of damage following SCI by ~25% and ~50%, whilst axonal damage, assessed by sera phosphorylated neurofilament-H levels, was significantly reduced following HFI-1 (~70%) and mexiletine (~30%) treatment ($P<0.05$). These data indicate that HFI-1 may be a potential neuroprotective drug for the treatment of SCI.

POS-MON-048

INVESTIGATING THE ROLE OF KINASE-DEPENDENT ACTIVATION OF EPHA4 IN SPINAL CORD INJURY

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PURPOSE Recent research has indicated that the EphA4 knockout (KO) mouse displays increased axonal regeneration and functional recovery following spinal cord hemisection. This occurs in association with reduced gliosis, a process that is thought to inhibit axonal regeneration after injury. In normal animals, Rho and MAP kinase activation through EphA4 may lead to the activation of astrocytes thereby inducing gliosis. If this is true, then inactivating only the kinase domain of EphA4 should produce the same outcome as the complete EphA4 KO. **METHOD** Wildtype (n= 6), EphA4 kinase dead (EphA4KD) (n= 4) and EphA4KO (n=3) animals were anaesthetised using 2% isoflurane and the spinal cord hemisectioned in the thoracic region (T11-T12). Five weeks following injury, animals were again anaesthetised and the spinal cord exposed at the cervical level C5. Fluoro-ruby tracer dye was injected at 3 points and the animals allowed to recover. One week later they were perfused, and 60µM longitudinal sections of the spinal cord were cut, mounted and imaged using confocal microscopy. In another experimental series the spinal cord of wildtype, EphA4KO and EphA4KD animals was hemisectioned at T11-T12. The animals were sacrificed 4 days after injury and spinal cord tissue processed for immunoblotting (n=3 in each group) and immunohistochemistry (n=3 in each group). **RESULTS AND CONCLUSIONS** As previously published, the EphA4 KO animals displayed axonal regeneration, functional recovery and decreased expression of the astrocytic marker glial fibrillary acidic protein (GFAP) following spinal cord hemisection. In contrast, the EphA4KD mouse did not display axonal regeneration although axons did regenerate closer to the lesion site than in wildtype animals. Immunoblots demonstrated that GFAP expression in the EphA4KD mouse was similar to that seen in wildtype mice. Taken together these results suggest that EphA4 does not work primarily through its kinase domain to inhibit axonal regeneration and induce gliosis.

POS-MON-049

A BREACH IN THE DURA MATER AFFECTS SCAR FORMATION AND COMPOSITION AFTER SPINAL CORD INJURY

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Purpose: After spinal cord injury a scar forms, which creates a physical and biochemical barrier which is impenetrable to regenerating axons. The composition and severity of this scar may vary depending on the ability of extrinsic cells to enter the injury site. In this study we aim to determine if a breach in the dura mater changes the composition and severity of scar formation. **Methods:** 12 week Female F344 rats were divided into groups of 8 and given either: a contusion injury (intact dura group), a contusion injury where the dura was torn or a dorsal hemisection (dural breach groups) injury at T8. After 4 weeks the severity and composition was determined with Masson's trichrome staining, GFAP and rPH immunostaining. **Results:** There was no collagen or fibroblast staining was observed in the contusion only rats, and very little GFAP+ astrocyte staining within the wound. In the contusion rats where the dura was breached, the spinal cord had adhered to the outside tissue and there was a little collagen and fibroblast staining within the wound, but a more intense GFAP+ astrocyte staining than the contusion alone. In the hemisection spinal cords there was a much greater adherence to the external tissue and large amounts of collagen staining in the wound and distal to the injury, the greatest intensity of GFAP+ astrocytes around the margins of the wound, but still very little fibroblast staining.

POS-MON-050

IS THE EXCITABILITY OF SINGLE CUTANEOUS AFFERENT C FIBRES MODIFIED FOLLOWING SPINAL CORD INJURY IN THE RAT?

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Purpose: Cutaneous afferents responsible for thermal and nociceptive sensations are small diameter slowly conducting myelinated (A δ fibres) and unmyelinated axons (C fibres). Our recent ultrastructural analysis of the rat sciatic nerve revealed that unmyelinated axons had atrophied by 10-15% at 8 weeks after spinal cord injury [1]. In the present study, we have investigated whether these changes in axonal dimensions are associated with conduction abnormalities. **Methods:** Extracellular recordings have been made from single C fibres of the sural nerve of rats anaesthetized with pentobarbitone (45-50 mg/kg i.p.) and the conduction velocity (CV) determined from the latency of the response following stimulation of L5 dorsal root. **Results:** In intact control rats, C fibre CV ranged from 0.5 to 2 m s-1 with a median value close to 1 m s-1. Similar recordings on spinalized and sham-operated rats (n≥7 in each group) yielded CVs with a similar range and median. Analysis of multiple successive sweeps suggests that abnormalities of conduction such as multiple firing to a single stimulus and latency jitter of slowly conducting C fibres are more common in spinalized rats than in intact rats. **Conclusion:** The data suggest that changes in excitable properties may be associated with axonal atrophy in cutaneous afferents following spinal cord injury. Our findings also provide additional basic information about the membrane properties of unmyelinated axons. 1. Kettle EK & McLachlan EM. (2004). Proceedings of the Australian Neuroscience Society, p. 114.

POS-MON-051

DELAYED TRANSPLANTATION OF HUMAN OLFACTORY ENSHEATHING CELLS IMPROVES LOCOMOTOR RECOVERY FOLLOWING A MILD CONTUSION INJURY IN ATHYMIC RAT

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Transplants of olfactory ensheathing cells (OEC) into spinal cord lesions have been shown to enhance recovery in several different animal models. **Purpose:** This study tested the effect of human OEC transplantation on locomotor function following a mild spinal cord injury in athymic (Nude) rats. Nude rats were used to prevent immune rejection of the human cells. **Methods:** hOEC were isolated and purified (> 95%) from adult human donors and genetically labelled with green fluorescent protein using a lentiviral vector. 20 rats were subjected to a mild contusion injury (12.5mm weight drop). Acutely (n=12), or at 1 week delay after injury (n= 8), 1 million hOEC, or DMEM culture medium alone, was injected into the epicenter of the lesion (5ul) and 1mm rostrally and caudally into the adjacent spinal cord (2 x 1ul). Behavioural tests were carried out weekly for 6 weeks to assess locomotor function and histology was undertaken to examine tissue damage. **Results:** hOEC survived for up to 6 weeks in injured cord. Delayed injections resulted in a marked improvement of hindlimb function in the hOEC treated animals using the BBB scoring method compared to DMEM treated animals and to acute injections (repeated measures ANOVA, $p < 0.05$). **Conclusions:** Using an athymic model of mild SCI we have shown that hOEC survived in the cord and improved locomotor function when transplanted at 1 week after injury but failed to do so when transplanted at the time of injury. The cellular mechanisms for these improvements remain to be investigated.

POS-MON-052

TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS OF THE VLPAG ARE ACTIVATED BY NERVE INJURY AND ARE IMMUNOREACTIVE FOR CCK RECEPTORS

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Constriction injury of the sciatic nerve evokes alterations to the sleep-wake cycle in a sub-population of rats (~30%) despite all animals displaying the sensory changes characteristic of neuropathic pain. Neuroplastic changes which might underlie the expression of altered sleep wake cycle behaviours include a select increase in the expression of cholecystokinin in the ventrolateral periaqueductal gray (vlPAG). It has been shown that tyrosine hydroxylase (TH-) immunoreactive neurons in the vlPAG which project to the hypothalamus are critical for the transition from wakefulness to sleep states. It is possible therefore that the alterations in sleep-wake cycle seen in nerve-injured rats might be triggered in part by an action of CCK-8 on TH-IR vlPAG neurons. To address this question we utilised single and double-label immunofluoresence studies to determine the location of CCK-receptors on TH immunoreactive neurons and to determine the effects of nerve injury on vlPAG, TH expression. Rats (N=10) were deeply anaesthetised and perfused with fixative and standard immunohistochemical techniques used to reveal TH- and CCK receptor immunoreactive neurons. TH-IR neurons were counted in six equidistant sections through the PAG (mean 62± 9 TH-IR neurons) and the number of TH- and CCK receptor IR neurons determined. Eighty seven percent of the TH-IR neurons contained CCK receptors. To determine the effects of nerve injury on these cells RT-PCR for TH mRNA was performed on the PAG of injured rats with and without sleep disturbance (N=16). Compared to controls, expression of TH increased by 34±6% following nerve injury in both sets of rats. These data show: (i) that the majority of vlPAG TH-IR neurons contain CCK and; (ii) that nerve injury activates TH-IR vlPAG neurons. It is possible therefore that increased CCK following nerve injury might alter activity in these cells resulting in altered sleep behaviour.

POS-MON-053

PROJECTIONS FROM THE PERIAQUEDUCTAL GREY TO THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS

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It has been proposed that neurons of the paraventricular nucleus of the thalamus (PVTh) play an important role in regulating the activity of the hypothalamic-pituitary-adrenal axis (HPA) during chronic, but not acute stress. This regulatory activity appears to be independent of the conventional negative feedback system determined by plasma corticosterone levels. The activity of the PVTh in response to chronic stressors is also suggested to be regulated by a cholecystokinin-ergic input which arises from ponto-mesencephalic sources. In light of our recent findings that cholecystokinin is up-regulated in the periaqueductal grey region (PAG) of rats expressing dysfunction of the HPA axis following nerve injury, we aimed to determine (i) whether PAG neurons project into PVTh regions (posterior PVTh) known to influence the HPA axis and (ii) whether these neurons contained glucocorticoid receptors and could be directly modulated by corticosterone. The retrograde tracer Cholera toxin B was injected into the PVTh of sixteen rats at anterior (N=4), intermediate (N=5) and posterior (N=7) levels. Seven days later each rat was deeply anaesthetized, perfused with fixative and the brain removed. Serial coronal sections were taken and single label (CTB) and double label (CTB and glucocorticoid receptor) immunohistochemical procedures were performed to reveal the location within the PAG of PVTh projecting neurons. The anterior PVTh was targeted primarily by an ipsilateral projection arising from the intermediate portion of the dorsolateral PAG whereas the posterior PVTh was targeted by an ipsilateral projection arising from the caudal ventrolateral PAG. Whilst glucocorticoid receptor containing neurons were in abundance in the PAG, PVTh-projecting PAG neurons were never double labeled. These data suggest that ventrolateral PAG neurons are well placed to play a role in the corticosterone-independent regulation of the posterior PVTh during chronic stressors.

POS-MON-054

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

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Chronic neuropathic pain is characterised by both sensory and affective changes. We have shown that following sciatic nerve constriction injury (CCI), 30% of rats develop a persistent change in their complex behaviours (social interaction, sleep-wake cycle and motivated behaviours) identical to chronic neuropathic pain patients. We have also demonstrated that an animal's intrinsic coping style to physical and/or psychological stressors predicts the development of this pattern of disability following nerve injury. Rats, which fail to adopt a consistent coping style (i.e., either proactive or reactive) are most vulnerable to the development of disabilities after injury. The periaqueductal grey region (PAG) has been shown to be a brain region critical for the expression of emotional coping behaviours, in these experiments we aimed to determine whether rats with proactive, reactive or "shifting" coping styles were characterised also by specific patterns of gene expression in the PAG. Our genes of interest in the first instance were those known to be selectively regulated by CCI: BAX/Bcl2, CAMK2B, CB1, CCK, CD200, GFAP, SYNJ2 and Vimentin. Rats (N=32) were characterised as either, proactive, reactive or shifting using 25 behavioural criteria, in a well characterised behavioural test battery. RT-PCR was used to determine gene expression levels in the PAG following behavioural testing, compared to an un-tested "control" population of age/weight/strain/litter matched rats (N=64). Significantly higher levels of expression of CB1, CCK and GFAP characterised proactive rats, and significantly lower levels of BAX:Bcl2 CB1, CCK and Vimentin characterised reactive rats. Rats with a shifting coping style did not differ significantly from the control population. Taken together with the earlier observation that it is the "shifter" rats which show high vulnerability to developing disabilities, and which show select regulation of the genes of interest, the degree of gene regulation after CCI is more dramatic than first appreciated.

POS-MON-055

ALTERATIONS IN HYPOTHALAMIC GLUCOCORTICOID RECEPTOR AND CORTICOTROPIN RELEASING FACTOR MRNA LEVELS IN RATS WITH NERVE INJURY-EVOKED DISABILITY AND PAIN

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Peripheral nerve injury evokes disabilities in ~30% of rats, despite all animals displaying sensory changes characteristic of neuropathic pain. Disabled rats (like chronic pain patients) exhibit disruption of the hypothalamo-pituitary-adrenal (HPA) axis, characterised by decreased plasma ACTH and increased plasma corticosterone (Cort). The negative feedback relationship between Cort and ACTH levels in the plasma is supported by the finding in disabled rats of an up-regulation of the numbers of glucocorticoid receptor (GR) and corticotropin releasing factor (CRF) immunoreactive (-ir) neurons in the paraventricular hypothalamic nucleus (PVN), a critical pituitary-regulatory region. Further, it has been shown that in disabled animals there is a down-regulation of numbers of GR-ir neurons colocalised with CRF-ir neurons in the PVN compared to rats with no disability. We therefore sought to investigate further these changes occurring in the PVN by identifying the patterns of the mRNA coding for GR and CRF within the hypothalamus, and how this may be correlated with the expression of disability. Rats were given a sciatic nerve constriction injury and evaluated post-injury for the presence of complex behavioural disabilities. Six days following nerve injury, rats (N=24) we decapitated and the hypothalamus isolated. RNA was extracted from the fresh tissue and used to perform real time PCR for the genes encoding GR and CRF. In both disabled and non-disabled animals GR mRNA increased significantly (+1.16 and +1.48 fold respectively). CRF mRNA showed a significant decrease in animals with disability (+ 0.93 fold) and a significant increase in animals with no disability (+1.11 fold). Evidently, nerve injury evokes changes in hypothalamic mRNA levels of GR and CRF in both disabled and non-disabled rats. These results may suggest differences in the mechanisms underlying the regulation of mRNA levels of GR and CRF (crucial to HPA axis functionality) according to the expression of disability.

POS-MON-056

FURTHER EVIDENCE IN THE RAT FOR CHOLECYSTOKININ-DEPENDENT MECHANISMS IN THE DISABILITIES TRIGGERED BY SCIATIC NERVE INJURY

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Constriction injury of the sciatic nerve evokes behavioural disabilities in only a sub-population of rats (~30%) despite all animals displaying the sensory changes characteristic of neuropathic pain. Neuroplastic changes which might underlie the expression of disabilities include a large and specific up-regulation of the mRNA for cholecystokinin, as well as an anatomically restricted expression of its translation product, the neuropeptide CCK-8 in the periaqueductal gray (PAG) region. Rats with behavioural disabilities and pain were characterised by dense terminal labelling located in the ventrolateral and the medial portion of the lateral PAG. Rats with pain, but no disability showed little terminal immunoreactivity. These data raise two important questions: Firstly, do PAG neurons possess receptors for this neuropeptide transmitter? Secondly what are the likely anatomical sources of the increased CCK-IR? (i) CCKA & CCKB receptors were localised in serial sections of the PAG using fluorescence immunohistochemistry. Receptor immunoreactive cells were counted in five equidistant sections from six rats. CCKA receptors were the most numerous in the caudal third of the PAG in both the lateral and ventrolateral regions. CCKB receptors showed identical distribution but were fewer (~30%) in number. PAG neurons in regions of injury-evoked increases in CCK-8 have the receptor profiles to respond to the increased release of CCK. (ii) A combination of retrograde tracing and immunohistochemical detection of CCK-8 was used to determine the potential sources of nerve injury increased CCK-8 in the PAG. Tracer injections were made into the lateral (N=6) and ventrolateral PAG (N=5). The dorsomedial subnucleus of the NTS, was a region rich in CCK-8-IR cell bodies, it also contained retrogradely-labelled cells, however no double-labelled neurons were detected. These data suggest strongly that the NTS is unlikely to be a source of increased CCK-8 in the PAG of rats with disability and pain.

POS-MON-057

A QUANTITATIVE STUDY OF THE EXPRESSION OF SORTILIN AND P75 RECEPTORS DURING POST-NATAL DEVELOPMENT IN THE MOUSE SPINAL CORD

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Purpose: Sortilin and p75 act as co-receptors for the pro-neurotrophin induction of cell death. Recently, sortilin and p75 were implicated in corticospinal motoneuron apoptosis after lesioning. The purpose of this study was to show that these receptors are expressed in spinal motoneurons and additionally to quantify and compare this expression during early post-natal development to adulthood. **Methods:** Sortilin and p75 expression were examined by immunohistochemistry in spinal cord from Balb/C mice and quantified by western blots in homogenates taken from the lumbar region of mice (n=3), 1, 4, 8 and 60 days after birth. **Results:** Results show that both sortilin and p75 are expressed in spinal motoneurons at post-natal day 1 (PND-1), with only sortilin expression continuing into adulthood. Furthermore, in the mouse spinal cord between PND-1 and PND-60, relative to total protein, the level of expression of sortilin increases by 39%, whereas p75 expression decreases by 65%. These results demonstrate a reciprocal level of expression of sortilin and p75 receptors during this developmental period. **Conclusion:** It is concluded that both sortilin and p75 are expressed *in-vivo* in the neonatal and adult mouse spinal cord, but not at a constant ratio. As co-expression of these receptors is necessary for pro-neurotrophin mediated apoptosis, a decreasing level of expression of p75 in comparison to sortilin in motoneurons during later development and into adulthood may help explain the higher susceptibility of neonates to neuronal cell death after axotomy.

POS-MON-058

P75 ANTISENSE INFUSION INCREASES HIPPOCAMPAL CHAT ACTIVITY IN NORMAL RATS

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Studies of the p75 knockout mouse suggest that p75 is a potent inhibitory regulator of the septo-hippocampal system. To test this, we administered antisense and nonsense oligos targetting the p75 neurotrophin receptor by intra-cerebral infusion for 4 weeks in normal rats. The oligos were delivered into the vertical diagonal band of Broca (VDB). The oligos were gapmer constructs consisting of discrete, adjoining phosphorothioate (PS) DNA and methoxy-RNA segments. Infusion of p75 antisense gapmers at 22 mcg/day increased hippocampal ChAT activity by 39% (n = 5), and infusion at a dose of 9 mcg/day increased hippocampal ChAT activity by 17% (n = 7). Infusion of gapmer oligos at 3 mcg/day had no effect. Infusion of morpholino antisense oligos (n = 8) and conventional PS antisense oligos (n = 6) did not produce significant changes in hippocampal ChAT activity. We measured p75 mRNA levels in the septum/VDB region, by quantitative real-time PCR. The results showed a strong correlation between p75 mRNA suppression and ChAT activity: p75 mRNA was strongly suppressed by the gapmer antisense treatments, suppressed to a lesser extent by morpholino antisense and least of all by the PS antisense treatment.

POS-MON-059

FRET ANALYSIS OF INTERACTIONS BETWEEN THE P75 NEUROTROPHIN RECEPTOR AND ITS CO-RECEPTORS

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The p75 neurotrophin receptor is a multifaceted type I transmembrane receptor for the neurotrophin family of ligands. p75 can signal either alone or in conjunction with co-receptors, such as the tropomysin receptor kinases (Trk) and sortilin, to mediate a diverse array of effects including cell death (acting alone or complexed with sortilin) or survival (with TrkA). We hypothesized that hetero- and homodimer interactions between p75 and its co-receptors would correlate with function and, in particular, that preventing dimerisation of p75 would block cell death. To begin to test this idea, we employed fluorescence resonance energy transfer (FRET) using N- and C-terminally tagged p75 fluorescent protein fusion constructs expressed in HEK293 cells. Surprisingly, our results revealed that, regardless of the presence or absence of neurotrophins or co-receptor expression, the intracellular domains of two p75 molecules are in close enough proximity to each other to cause FRET. By contrast, FRET between the extracellular domains does not occur under any of these conditions. Since activation of p75 signals requires proteolytic removal of the extracellular domain, we also investigated the level of FRET when cleavage of p75 was either induced or prevented. Although the dimeric state of p75 was unaltered by its proteolysis, these data, together with those from experiments using a series of deletion and mutant p75 constructs, suggest the region of interaction between p75 molecules is located around the transmembrane domain region. Mutations within this domain of p75 which prevent its dimerisation will next be tested for their ability to interact as monomers with co-receptors of p75 as for their ability to mediate death signaling.

POS-MON-060

NEUROTROPHINS REGULATE THE TRAFFICKING DYNAMICS OF P75NTR

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Purpose: To examine whether neurotrophins (NTs) individually regulate the internalisation and trafficking of neurotrophin receptor p75 (p75NTR) in the NSC-34 motoneuron cell line, with the aim of increasing our understanding of dynamic vesicle trafficking of p75NTR. **Methods:** Neurotrophic factors, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 and 4 (NT-3 & NT-4) were used in conjunction with a fluorescently labelled monoclonal antibody to p75, to examine neurotrophin specific differences in p75NTR internalisation rates, endocytotic mechanisms and subsequent cytoplasmic localisation using live and fixed confocal microscopy. **Results:** NGF and NT-3 treatment of NSC-34 cells leads to apoptosis, but BDNF and NT-4 treatment does not (n=4). Further, p75NTR internalisation rates are altered in the presence of NGF, NT-3, or NT-4, but not BDNF (n=8), and the receptor is diverted into non-CME pathways in response to NGF but not BDNF (n=6). Immunofluorescence confocal microscopy suggests that p75NTR equally recycles to the plasma membrane in a Rab4 dependent manner, or is degraded in lysosomes, in the absence of NTs (n=10). The addition of neurotrophins diverted p75NTR from the recycling Rab4 positive pathway, into EEA-1 positive sorting endosomes in the presence of NGF or NT-3 (n=4), or lysosomes in the presence of BDNF or NT-4 (n=4). **Conclusion:** These findings clearly demonstrate that p75NTR internalisation followed by sorting to endosomal, recycling or lysosomal pathways, depends on the type of neurotrophin present.

POS-MON-061

SORTILIN AND THE P75 NEUROTROPHIN RECEPTOR ARE NOT CO-EXPRESSED IN THE CELLS AND TISSUES OF THE NORMAL HUMAN IMMUNE SYSTEM

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Purpose: The neurotrophin receptor, p75NTR (or CD271), is a member of the Tumor Necrosis Factor receptor (TNFR) super family of transmembrane proteins that binds pro and mature forms of the neurotrophins. Recent studies have provided evidence that the pro-forms of neurotrophins transmit cell death signals by binding receptor complexes consisting of CD271 and sortilin, (a member of the Vs10p-domain receptor family). Abnormal expression of CD271 also has been associated with allergy, various neuronal and non-neuronal tumors and implicated in malignancies such as chronic lymphocytic leukemia. In this study we sought to determine the expression pattern of CD271 and sortilin in the immune system, specifically addressing whether they are expressed in the same cells. **Methods**: The human lymphoid organ, the palatine tonsil, was examined for p75 and sortilin expression by immunohistochemistry and human peripheral blood lymphocytes (PBL) using flow cytometry. Results were confirmed using western blot analysis. Monoclonal anti-human p75NTR (clone MLR2), polyclonal anti-human p75NTR and rabbit polyclonal and mouse monoclonal sortilin antibodies were used for these studies. **Results**: CD271 and sortilin were expressed in distinct areas of human palatine tonsil tissue, with p75 confined to the germinal centre and most sortilin staining outside this region. PBL analysis by flow cytometry indicated sortilin is not co-expressed with CD271 in leukocytes. This finding was confirmed by western blot of sorted cells. **Conclusion**: Although sortilin is a co-receptor with p75 for pro-neurotrophin-mediated neuronal death, this current study found no evidence for co-localization of the two receptors in the cells and tissues of the normal human immune system.

POS-MON-062

THE INFLUENCE OF CORTICAL BETA OSCILLATORY ACTIVITY ON MOTOR EVOKED POTENTIAL VARIABILITY IN HUMANS

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Purpose: Transcranial magnetic stimuli (TMS) applied to the human motor cortex evoke electromyographic (EMG) responses in contralateral hand muscles, known as motor evoked potentials (MEPs). The trial by trial amplitude of MEPs is highly variable. Reasons for this variability are not fully understood but modulations of cortical excitability due to oscillatory activity may be important. Beta frequency oscillatory activity (13-30 Hz) is influenced by motor activity. Here we examined whether changes in beta frequency electroencephalographic (EEG) activity might contribute to MEP variability. **Methods**: EEG recordings were made from normal subjects (n=12) with a pair of Ag/AgCl electrodes, one over the motor hand area and the other over Fz. EMG recordings were made from the right first dorsal interosseous muscle (FDI). Single pulse TMS was applied with an intensity sufficient to evoke MEPs of 0.5-1 mV in the relaxed FDI. Subjects were studied 3 times on different days. On each occasion a 30 s EEG epoch was recorded with the subjects' eyes closed. Approximately 1 minute later 20 MEPs were recorded. A 15 s epoch of artefact free EEG was then analysed using fast fourier transform. The beta frequency band power was correlated against the coefficient of variation of MEP amplitude for each testing occasion. **Results**: Regression analysis revealed a highly significant ($p = 0.0028$) 2nd order polynomial correlation ($R=0.55$) between the MEP coefficient of variation and beta power. Relatively low and high levels of beta power were associated with greater variability than moderate levels of beta power. **Conclusion**: This result suggests that ongoing modulation of beta power may be a significant cause of MEP variability.

POS-MON-063

AUTOREGULATION OF CORTICAL INHIBITION EXPLORED WITH PAIRED-PULSE TRANSCRANIAL MAGNETIC STIMULATION (TMS) OF HUMAN MOTOR CORTEX

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Introduction GABAergic inhibitory synapses in motor cortex exert their effect through post-synaptic receptors (GABAa and GABAb) that mediate short-and long-interval cortical inhibition respectively, and a pre-synaptic autoreceptor that inhibits further GABA release and temporarily reduces the efficacy of the synapse. Post-synaptic inhibition has been well studied with paired-pulse transcranial magnetic stimulation (TMS) protocols. In the present study we have used experimental evidence that the time-course of autoreceptor activation outlasts that of the post-synaptic receptors, to hypothesize that there will be a period of motor evoked potential (MEP) facilitation following long-interval cortical inhibition. **Methods** In 10 healthy right-handed subjects (19-36 years of age), MEP amplitude was measured from the right first dorsal interosseous (FDI) muscle following paired-pulse (conditioned-test) TMS delivered (every 5 secs) at inter-pulse intervals (IPIs) in the range 100-270ms (each interval repeated pseudo-randomly 6 times). The intensity of the test stimulus was set to give a MEP of ~0.5-1mV in amplitude, and the same intensity was used for the conditioning stimulus. Average test MEP amplitude for each IPI was expressed as a percentage unconditioned control. All recordings were made at rest. The duration of the cortical silent period (SP) to a single-pulse at the same intensity was measured during a low-level (~10%) voluntary contraction. **Results** Test stimulus MEP amplitude was reduced by up to 60% at IPIs of 100ms and 150ms ($p<0.05$) but returned to control at an IPI of 170ms. For longer IPIs there was a period of facilitation (~50%) between 190-210ms ($p<0.05$) after which MEP amplitude approached control again. SP duration was 181±5ms. **Conclusion** The activation of cortical inhibitory circuits with TMS is followed by a period of MEP facilitation, consistent with a reduction in the efficacy of inhibitory synapses through pre-synaptic autoregulation. Further studies are required to more directly probe cortical inhibitory efficacy during this period of facilitation.

POS-MON-064

INHIBITION OF THE TENDON REFLEX FOLLOWING ELECTRICAL STIMULATION OF TENDON AND CUTANEOUS AFFERENTS

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Transcutaneous tendon electrical stimulation and cutaneous nerve stimulation produce a similar pattern of strong reflex inhibition of the ongoing voluntary EMG activity in both heads of the human gastrocnemius muscle (GA). This inhibition may be due to postsynaptic inhibition of motoneurons or presynaptic inhibition of excitatory inputs to motoneurons. We investigated the nature of the inhibition at each site by comparing the time course of the inhibitory effect on the tendon reflex (TR) with the time course of voluntary inhibition. Healthy volunteers (n=8) were seated upright in a chair with the left lower limb securely attached to a frame. Ankle, knee and hip joints were kept at 90deg throughout the experiment. Bipolar surface EMG electrodes were placed 2 cm apart over the two heads of GA. A stimulus intensity of 60 mA was used to obtain tendon reflex inhibition. The GA tendon was stimulated using small metal plates located on the midline and adjacent to the musculotendonous junction and the adjacent anterior surface of the leg. Cutaneous afferents from the sural nerve were stimulated below the fibular malleolus. All shocks were constant current stimuli of 0.2 ms duration and maximum intensity of 35 mA. A linear motor provided brief taps to the GA tendon. Alternate resting TRs were paired with either sural nerve or tendon conditioning stimuli. Cutaneous conditioning inhibited the TR over an interstimulus interval (ISI) of 60 to <100 ms. The maximum strength of inhibition was 88.17+/- 4.36 %. Facilitation occurred in the interval 120-155 ms of mean strength 21+/- 8.34%. Tendon conditioning inhibited the TR over an ISI of 0 to <350 ms. The mean strength of inhibition was 94.35 +/- 2.51%. No evidence of facilitation was seen after tendon conditioning. When compared with cutaneous effects, the much longer time course of inhibition and absence of facilitation suggest that tendon conditioning effects are mediated by a different, possibly presynaptic, mechanism. The cutaneous effects, based on these observations and previous studies, are consistent with a postsynaptic mechanism.

POS-MON-065

NEUROBIOMECHANICAL MARKERS OF DISABILITY FOLLOWING RHEUMATOID ARTHRITIS

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Introduction: The nature and cause of the disability following rheumatoid arthritis remain unclear and objective measures of factors that cause disability are needed. Patients complain that joint stiffness restricts mobility but increased joint stiffness has not been experimentally observed. We studied metacarpophalangeal joint stiffness and the correlated reflex activity under passive conditions and over a range of isometric *first dorsal interosseus* (FDI) contraction in patients (n=10) and compared this data in normal subjects (n=10). **Methodology:** The index finger was rotated about the metacarpophalangeal joint by a computer-controlled servomotor. A broadband perturbation of the joint (1.0 - 45.0Hz) was used to assess joint stiffness and the muscle reflex response at rest and during constant contractions. Joint position was measured by a precision potentiometer and surface EMG was recorded from FDI. The force exerted by the finger was measured by a load cell and normalised for volume of the index finger in each subject. **Data Analysis :** The rectified and low-pass filtered (60Hz) EMG and torque signals were cross-correlated with the joint angle (stretch) data. **Results:** The torque gain was significantly increased ($p<0.05$) in patients during contraction but not at rest only when using perturbations of 1-5 Hz. However reflex gain was not significantly different ($p=0.11$) to controls in this frequency band at any contraction level. A significant reduction (~ 40%, $p<0.05$) in reflex gain was seen only during contraction and in the frequency band 45 Hz. The patient group also had a reduced maximum voluntary contraction (patients = 61.6 ± 39.7 μV, controls = 192.4 ± 69.9 μV), indicating pronounced weakness. **Conclusion:** There was no evidence of increased passive joint stiffness at rest. The increased stiffness seen during contraction was not due to reflex activity. Possible factors contributing to disability are weakness and increased viscous stiffness of joints or muscle during contraction.

POS-MON-066

GENERALIZED NEURONAL HYPER-EXCITABILITY AND ACCELERATED POSTNATAL DEVELOPMENT IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Transgenic mice over-expressing the G93A mutation of the human Cu-Zn superoxide dismutase 1 gene (hSOD1G93A mice) are a common animal model of amyotropic lateral sclerosis (ALS), showing biochemical and ultrastructural changes in motor neurons (MNs) by 2 months and selective degeneration and death of MNs at 3-4 months. We made whole cell patch clamp recordings from hypoglossal (XII) MNs and superior colliculus interneurons (SINs) in brain slices from anaesthetised (Na pentobarbitone, 100 mg/kg i.p.) wild type (WT) and hSOD1G93A mice aged 4-12 postnatal days. The frequency and amplitude of spontaneous (n=12) but not quantal (n=8) glutamatergic EPSCs and GABAergic IPSCs (n=7) was increased in hSOD1G93A SINs cf. WT SINs (n=17,7,4). NMDA EPSCs decayed faster (n=5) and were insensitive to the NR2B-selective blocker ifenprodil (n=3) in hSOD1G93A SINs from P10-12 mice, indicating loss of NR2B subunits at an earlier age than in WT SINs (n=7,3). Firing rates of hSOD1G93A SINs (n=8) were significantly higher than WT SINs (n=7). Similarly, hSOD1G93A XII MNs showed higher frequency and amplitude of spontaneous (n=5) but not quantal (n=7) glutamatergic EPSCs and glycinergic IPSCs (n=7) and faster decaying NMDA EPSCs (n=4) cf. WT XII MNs (n=10,5,4,4). Firing rate and persistent Na+ current were significantly larger in hSOD1G93A (n=6) cf. WT XII MNs (n=11). Retrogradely labelled hSOD1G93A XII MNs had significantly fewer contralaterally projecting dendrites at P6 than WT XII MNs, which retract these dendrites by P9. hSODG93A mice (n=11) showed transient deficits in forelimb placing and righting response at P2-3 and P2 cf. WT mice (n=31). These data suggest that the hSOD1G93A mutation induces a generalized neuronal hyper-excitability and accelerated neuronal development long before the appearance of biochemical, ultrastructural or death of MNs in an animal model of ALS.

POS-MON-067

THE UNFOLDED PROTEIN RESPONSE IS INDUCED IN SPORADIC ALS

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Purpose Sporadic and familial ALS have an identical clinical presentation, suggesting a common pathological mechanism. We showed recently that the unfolded protein response (UPR) was induced in transgenic SOD1G93A mice at symptom onset and disease end stage. However, although transgenic animals expressing human mutant SOD1 proteins are an invaluable disease model, SOD1-linked FALS represents only a small proportion of all ALS patients. Hence, we wanted to determine if UPR induction also occurs in human patient spinal cords, and whether ER stress is associated with sporadic disease or not. **Methods** Human patient lumbar spinal cord extracts were examined by western blotting and immunohistochemistry for the up-regulation of UPR markers in comparison to normal controls. **Results** Endoplasmic reticulum (ER) stress sensor kinases, chaperones and apoptotic effectors were all up-regulated in human ALS patient spinal cords. Furthermore, the ER chaperone protein disulphide isomerase (PDI) co-localised with protein inclusions in the remaining patient motor neurons and was up-regulated in the cerebrospinal fluid. **Conclusion** Our findings implicate ER-stress in the pathology of the much more common sporadic forms of ALS as well as familial ALS, thus placing the UPR centrally in the pathogenesis of a wide spectrum of motor neuron diseases. Further studies are required to establish how early in pathogenesis these events occur and what the primary triggers are.

POS-MON-068

METALLOTHIONEIN MEDIATED NEUROPROTECTION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Purpose: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons of the cortex and spinal cord. Mutations to the antioxidant enzyme, superoxide dismutase 1 (SOD1), have been linked to some familial forms of ALS. We predict that the neuroprotective metallothionein IIA (MT-IIA) protein may ameliorate some of the toxic gain-of-function effects of mutant SOD1 in the G93A SOD1 transgenic mouse. **Method:** At 10 weeks of age, litter pairs comprising a wild type and a mutant G93A SOD1 mouse, were injected with either MT-IIA (n = 9) at 10µg/10g body weight or a saline control (n = 8) at 10µL/10g body weight twice a week until the mice reached endstage, a loss of 20% maximum body weight. At each injection time point, weight and symptoms (tremors, hind limb mobility and muscle wastage) were assessed. These data were compiled together and a group analysis was performed. **Results:** Analysis of symptom progression over the course of the experiment revealed a delay in neurodegenerative symptom development in the MT-IIA treatment group beginning at approximately 137 days of age. This preceded the deviation observed in survival between the two treatment groups, which occurred at approximately 147 days of age. Overall we observed an increase in survival of the MT-IIA treated group by 6%. **Conclusion:** These results suggest that the MT-IIA treatment can delay the severity of neurodegenerative symptoms and consequently improve survival in the G93A SOD1 mouse. Histological analysis is underway to determine if there are pathological differences within the sciatic nerve and spinal cords of mice between the treatment groups.

POS-MON-069

INHIBITORY RECEPTORS IN MOTOR NEURON SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Purpose: Amyotrophic Lateral Sclerosis is characterized by the selective death of upper and lower motor neurons. Our laboratory has reported differences in the levels of excitatory metabotrophic glutamate receptors in motor neuron pools vulnerable to degeneration compared to resistant motor neurons. In this study we have characterized the patterns and levels of inhibitory $GABA_A$ and glycine receptors in five different motorneuron pools, three that are vulnerable (facial, hypoglossal, ventral horn of the spinal cord) to degeneration in ALS and two that are resistant (oculomotor, abducens). **Methods:** Immunohistochemistry was performed on sections of long-term fixed human brainstem tissue taken from 5 cases obtained from the Neurological Foundation Human Brain Bank at Auckland University. Antibodies specific to the α and β subunits of the $GABA_A$ receptor and gephyrin, a protein that co-localises with glycine receptors, were employed. Results were analysed by light microscopy. **Results:** Specificity of antibodies was tested and the various immunohistochemical parameters for staining were determined. There is widespread, dense staining of all receptor subunits within the 5 motor nuclei investigated. Differences in the level of α subunit staining are seen between the hypoglossal (vulnerable) and abducens (resistant); there is also a significantly higher level of staining for glycine receptors within the hypoglossal nucleus compared to the abducens. **Conclusion:** These results show a pattern of staining similar to that obtained in the rat (Lorenzo et al., 2006) and provide further insight into why certain motor neuron pools may be more vulnerable to excitoxicity. Further work needs to be done to extend the analysis to other motor areas affected by ALS.

POS-MON-070

INHIBITION OF ENDOPLASMIC RETICULUM STRESS AND PREVENTION OF MUTANT SOD1 AGGREGATION BY PROTEIN DISULFIDE ISOMERASE

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Purpose: Endoplasmic reticulum (ER) stress is a feature of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Familial forms of ALS can be caused by over 130 different mutations to the gene encoding superoxide dismutase-1 (SOD1), which lead to disulfide reduction and accumulation of misfolded protein. We previously showed induction of ER stress and upregulation of protein disulfide isomerase (PDI), a molecular chaperone and disulfide bond modifying enzyme, in mutant SOD1 ALS models. Therefore, this study sought to identify whether or not PDI could prevent ER stress induction and inhibit aggregation of mutant SOD1. Methods: Stable motor neuron-like NSC34 cell lines overexpressing PDI were constructed and transiently transfected with SOD1-EGFP constructs. Immunocytochemistry and confocal microscopy were performed. The percentage of inclusion-positive cells was calculated by counting at least 500 SOD1 expressing cells per treatment. ER stress markers in cell lysates and insoluble SOD1 and ubiquitinated proteins were detected using semi-quantitative immunoblotting. Results: The percentage of inclusion positive cells was significantly decreased in cells overexpressing PDI compared to control, from ~15% to ~5% for mutant A4V and from ~10% to ~3% for mutant G85R ($p<0.001$, n=4). The amounts of insoluble mutant SOD1 and high molecular weight ubiquitinated proteins were also decreased. Levels of the ER misfolded protein binding protein BiP and apoptotic protein CHOP were lower in PDI overexpressing cells, and phosphorylation of the ER sensor PERK was similarly inhibited. Conclusion: Protein disulfide isomerase may play a neuroprotective role in ALS by preventing ER stress and correcting mutant SOD1 misfolding.

POS-MON-071

MATRIX METALLOPROTEASE-9 IS DECREASED IN SERUM OF TRANSGENIC SOD1G93A MICE AND AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset fatal neurodegenerative disorder characterized by progressive deterioration of motor neurons. It is clinically manifested by significant weight loss, muscle wasting, and spasticity leading to paralysis and eventually death through respiratory failure. Although the aetiology of this debilitating disease remains unclear, more than 100 mutations in the copper-zinc superoxide dismutase 1 (Cu-Zn SOD1) gene have been identified to cause familial ALS implicating a role of SOD1 in ALS pathogenesis. Transgenic mice overexpressing human mutant SOD1G93A (TgSOD1G93A) produce a phenotype that closely replicates both clinical and pathological hallmarks of human ALS. Purpose: Matrix metalloproteinase-9 (MMP-9) activity is proposed as a potential biomarker in ALS as its activity is altered in muscle and serum of ALS patients. However, studies have presented conflicting evidence on whether MMP-9 is up- or down-regulated in ALS. Therefore, we examined how MMP-9 levels are modulated in TgSODG93A mice and ALS patients. Methods: Zymography and Western Blot were used to measure MMP-9 levels in serum from both TgSOD1G93A mice with slowed disease progression and human ALS. Results: A significant decrease in MMP-9 activity and expression levels were observed in end stage (n=10) compared to pre-symptomatic (n=10) TgSOD1G93A mice. MMP-9 expression levels were also significantly decreased in ALS patients (n=17) (serum from VBBN) compared to the age-matched controls (n=14). These results suggest that MMP-9 expressions are reduced in the latter stages of ALS. More studies are required to determine the role of MMP-9 in the aetio-pathology of ALS.

POS-MON-072

GAIT DYNAMICS OF THE SOD1 RAT MODEL OF MOTOR NEURONE (MND) DISEASE USING DIGIGAIT™ IMAGING SYSTEM

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Introduction: Motor Neurone Disease (**MND**) is a progressive human neurodegenerative disorder, characterised by motor deficits that progress to paralysis and respiratory complications resulting in death. The SOD1 rat model of MND that has motor dysfunction is conventionally measured using rotarod. However motor dysfunction is not detected until 16 weeks even though motor neuron loss can commence at 8 weeks. It is likely that subtle weakness and spasticity may be first apparent in walking patterns. This study aimed to establish whether motor dysfunction could be identified earlier and more reliably using temporal and spatial indices of gait using DigiGait system than with the rotarod. Method: SOD1 and wild type (WT) rats were trained on the rotarod and then tested for 180s at 16 rpm. For DigiGait imaging analysis rats ran on a motorized transparent treadmill and ventral plane vidoegraphy is recorded. Software quantifies a range of gait indices. Rats were tested at 13 and 15 cm/s. Rotarod and gait dynamics were compared between SOD1 (n > 6) and WT (n > 6) rats aged 13 weeks. Results: Abnormal gait was detected in the SOD1 rats using the DigiGait system, while no differences were found on the rotarod. The main abnormality was a longer hind swing rate at both speeds in SOD1 (0.15 ± 0.009 s) than in WT (0.12 ± 0.007 s) rats. Conclusion: These results identify subtle motor dysfunction in SOD1 rat using DigiGait imaging analysis that is not evident when tested on the rotarod and which may be symptomatic of the early pathological changes that occur in the this model of MND disease.

POS-MON-073

IN VIVO DIFFUSION TENSOR MRI OF THE LUMBAR SPINAL CORD IN G93A-SOD1 MICE

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Purpose: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease (MND) in humans, characterised by selective degeneration of motor neurons. Currently, clinical diagnosis relies largely on behavioural tests or histological methods to directly count neurons in affected areas. Diffusion tensor imaging (DTI) can provide information on axonal organization and has been previously used to detect both axonal and myelin spinal cord damage. Here we examine the utility of DTI to measure degeneration in the lumbar spinal cord of the G93A-SOD1 transgenic mouse model of ALS. **Methods:** Spinal cord imaging of both affected SOD1 mice and wildtype littermates was performed using a 16.4 T spectrometer. *Ex vivo* imaging of paraformaldehyde-fixed spinal cords (125 days old, n=4) was acquired using diffusion sensitising gradients in six directions, with a resolution of 50 µm x 50 µm. Animals were imaged *in vivo* at ~100, 125 and 145 days of age (n=3), with a resolution of 70 µm x 70 µm. Fractional anisotropy (FA) values were obtained from regions of interest within the white matter of each lumbar spinal cord. Grip strength testing of hind limbs was used to monitor disease progression. **Results:** The FA values were reduced in the ventrolateral white matter of the lumbar spinal cord of ALS affected SOD1 mice compared to wildtype littermates, which became more pronounced with disease progression. In contrast, there was no difference in FA in the dorsal white matter of ALS affected SOD1 mice compared to wildtype littermates. **Conclusion:** DTI may provide a useful non-invasive method to monitor the progression of ALS, allowing earlier or more accurate diagnosis, as well as assessment of treatment efficacy.

POS-MON-074

CHARACTERISATION OF SOD1 POSITIVE INCLUSIONS IN RAT MODEL OF MOTOR NEURON DISEASE (MND)

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Aim: Our aim was to describe the distribution of SOD1 (Superoxide Dismutase 1) positive inclusions in the rat brain and spinal cord in the SOD1 model of MND. SOD1 protein is present normally within neurones, and SOD1 immuno-reactive inclusions can be detected in lumbar motor neurones late in the disease. **Methods:** Brain and spinal cord from wild-type and mutant (G93A mutant human SOD1 gene) rats at postnatal day 120 (n=2) were immuno-reacted with antibodies against human SOD1 protein, SMI-32 (dephosphorylated neurofilament H) and a nuclear marker (DAPI) and tagged with fluorescent secondary antibodies. Images were captured using a confocal microscope to assess the presence of SOD1 positive inclusions within wild-type and SOD1 animals. **Results:** SOD1 inclusions were present and most abundant in the lumbar spinal cord, and brain stem: in particular the Pontine reticular nucleus. In most neurones there was diffuse SOD1 immuno-reactivity throughout the cytoplasm of the cell. In the affected areas of SOD1 animals, inclusions were recognised as abundant punctate SOD1 immunoreactive aggregates. Cells bearing inclusions typically had abnormal nuclei or an enlarged space that presumably contained nuclear material.While inclusions were abundant in mutant rats, in wild-type rats SOD1 inclusions were identified but had normal appearing nuclei. **Conclusion:** Confocal analysis can be used to identify SOD1 inclusions throughout the brain stem and spinal cord. Further analysis is required to establish whether there are other differences, such as size, between inclusions of wild-type and SOD1 rats. A detailed analysis of the progression of these inclusions is important for an understanding of motor neurone death and disease pathogenesis.

POS-MON-075

IMMUNOHISTOCHEMICAL EXPRESSION OF TRYPTOPHAN-KYNURENINE PATHWAY METABOLITES IN AMYOTROPHIC LATERAL SCLEROSIS

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Microglia act as resident immune cells of the central nervous system and are putatively involved in conversion of L-tryptophan to quinolinic acid (QUIN) a neurotoxic metabolite of the kynurenine pathway (KP). We examined microglial activation, and immunohistochemical expression of indoleamine-2, 3 dioxygenase (IDO) (the first enzyme of the KP) and QUIN in cells of the motor cortex (MC) and spinal cord (SC) in human amyotrophic lateral sclerosis (ALS). ALS (n=4) and control (n=1) cases were processed to paraffin and cut at 7µm. Activated microglia were identified with HLA-DR antibody, while IDO and QUIN antibodies were used on sections pre-treated with 10µg proteinase-K in 5mM TRIS, pH 8.0. IDO was expressed in motor neurons of SC and neurons of the MC in ALS but not in control tissue. QUIN was expressed in glial cells and some motor neurons of the MC and ventral horn SC. The percentage of activated microglia in the MC and the lateral corticospinal tracts of the SC were significantly higher in the ALS cases (p<0.0001) compared to the control. This study has shown that immune-activated neurodegeneration is an important aspect of ALS as evident by 1) the degree of microglial activation, 2) the presence of the KP in motor neurons of the SC ventral horn and MC, and 3) expression of QUIN in some motor neurons of the ventral horn SC and MC. With no effective treatment for ALS potential therapeutic strategies that target the KP may hold the key to delaying the progression of this devastating disease.

POS-MON-076

EFFECT OF KYNURENINE PATHWAY INHIBITION ON NAD+ METABOLISM AND PARP ACTIVITY IN HUMAN BRAIN CELLS

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AIMS In the periphery, the kynurenine pathway (KP) is the principle route of L-tryptophan catabolism and NAD+ synthesis. However it is not known if KP metabolism is involved in NAD+ synthesis in the brain. We therefore assessed the effect of KP inhibition on NAD+ metabolism and PARP activity in human primary foetal astrocytes and neurons. **METHODS** The KP enzymes indoleamine 2,3-dioxygenase (IDO) and quinolinic acid phosphoribosyl transferase (QPRT) were inhibited by 1-methyltryptophan (1-MT) and phthalic acid (PA) respectively in primary cultures of human foetal astrocytes and neurons. Intracellular NAD(H) levels and PARP activity were quantified spectrophotometrically in cell homogenates. Cell viability was assessed by measuring lactate dehydrogenase (LDH) activity in culture supernatants. (n=4 for all experiments). **RESULTS** KP inhibition induced a dose dependent decrease in intracellular NAD(H) and concomitant increase in LDH activity in both astrocytes and neurons. Following treatment with the pro oxidant H2O2 and a KP inhibitor, astrocytes showed significantly reduced PARP activity compared to H2O2 treatment alone suggesting that an adequate intracellular NAD+ supply is essential for maximum PARP activation. Addition of either L-Tryptophan or the NAD+ salvage pathway substrate, nicotinic acid, to astrocytes treated with the KP inhibitor 1-MT restored NAD(H) concentrations to near normal levels. **CONCLUSION** KP metabolism is essential for maintaining NAD(H) levels and cell viability in both human primary foetal astrocytes and neurons. An adequate intracellular NAD+ concentration is necessary for PARP activation and hence efficient base excision repair of DNA.

POS-MON-077

CHARACTERIZATION OF THE KYNURENINE PATHWAY IN HUMAN PRIMARY NEURONS

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PURPOSE: The kynurenine pathway (KP) is a major route of L-tryptophan catabolism resulting in the production of neurotoxic, neuroprotective and immune tolerance-inducing intermediates. The KP has been shown to be involved in several neurodegenerative diseases. It is important to know what KP metabolites are produced by each brain cell types to be able to understand their interactions and to appropriately design and test therapies. We have previously characterized the KP in human macrophages, microglial cells and astrocytes. **METHODS:** In this study, we characterized the KP in human foetal neurons in comparison with the human neuroblastoma cell line SKNSH using RT-PCR, HPLC, mass spectrometry and immunocytochemistry. All experiments were done in triplicates. **RESULTS:** We found that neurons express all the KP enzymes but at different levels. Indoleamine 2,3 dioxygenase is strongly expressed by both primary neurons and SKNSH whereas kynurenine aminotransferase 2, kynureninase and kynurenine hydroxylase showed very low-level expression. Picolinic carboxylase was found expressed only in primary neurons, not in SKNSH. Because of this “late switch” SKNSH were able to produce low amounts of the excitotoxin quinolinic acid whereas primary neurons preferentially produced the neuroprotective picolinic acid. Moreover, primary neurons were to catabolize quinolinic acid. **CONCLUSION:** The net result of neuronal KP induction is therefore towards neuroprotection and immune tolerance. This study represents the first comprehensive characterization of the KP in neuron and also identified a new therapeutic target for brain tumours.

POS-MON-078

GLUTATHIONE PEROXIDASE AS A TARGET FOR METHYLMERCURY TOXICITY IN MOUSE BRAIN MITOCHONDRIA

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Purpose: Methylmercury (MeHg) is a highly neurotoxic agent which causes neurological and developmental deficits in animals and humans. It has been demonstrated that mitochondria represent a major target for MeHg toxicity. Oxidative stress plays a central role in MeHg neurotoxicity. In previous studies we demonstrated the inhibitory effects of mercury toward glutathione peroxidase – GPx (EC 1.11.1.9), a major endogenous antioxidant enzyme in the CNS. Here we investigated the role of GPx in MeHg-induced toxicity using mouse brain mitochondria. Methods: Twenty one adult male Swiss mice were orally intoxicated with a single dose of MeHg (40 mg/L in tap water) for 21 days. Another 21 animals were used as untreated controls. After treatment, brain mitochondria were isolated. Mitochondrial viability was assessed using the MTT assay; reactive oxygen species (ROS) formation was assessed using the DCFDA fluorescent assay; Lipid peroxidation (LPO) was assessed using the TBARS and FOX assays. The results are mean of three independent experiments undertaken in duplicate. Results: Pre-treatment of mice with MeHg caused a significant decrease in cytosolic and mitochondrial GPx activity. In parallel, MeHg caused a significant reduction on mitochondrial viability, which was accompanied by an exacerbated mitochondrial ROS formation. LPO was also increased after MeHg exposure. The incubation of mouse brain mitochondria with mercaptosuccinic acid, a potent inhibitor of GPx activity, significantly elicited MeHg effects toward the parameters analysed. In addition, the incubation of mitochondria with exogenous GPx completely reversed MeHg-induced mitochondrial lipid peroxidation. Conclusion: The results suggest that GPx is an important target for MeHg-induced neurotoxicity, being this enzyme crucial in counteracting MeHg pro-oxidative effects to brain mitochondria.

POS-MON-079

ANTI-MUSK MYASTHENIA GRAVIS PATIENT ANTIBODIES DISRUPT THE MOUSE NEUROMUSCULAR SYNAPSE

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Muscle Specific Kinase (MuSK) is a receptor tyrosine kinase crucial for clustering of acetylcholine receptors (AChRs) at the embryonic neuromuscular synapse. In classic Myasthenia gravis (MG) muscle weakness is caused by autoantibodies against the AChR, and impairment of the postsynaptic response to ACh. In a subset of MG patients, however, autoantibodies against MuSK (instead of AChR) have been recognised. Injection of these patient antibodies into 6-week old mice (45mg IgG /day over 14 days) caused progressive reductions in postsynaptic AChR packing densities in the tibialis anterior and diaphragm muscles, compared to mice injected with control IgG. Anti-MuSK injected muscles also showed (compensatory) increases in mRNAs encoding MuSK, AChR and rapsyn mRNA. Most remarkably, the presynaptic nerve terminal became misaligned from the postsynaptic AChR cluster. This misalignment suggests the breakdown of the normal relationship between pre- and postsynaptic elements. The severity of the synaptic changes varied with IgG from different anti-MuSK patients and with recipient mouse strain (C57Bl6 vs FVB). Mice with the most marked synaptic changes also displayed myasthenic symptoms including weight loss, muscle fatigue and decrement in the amplitude of the compound muscle action potential during repetitive nerve stimulation at 3Hz. We propose that ongoing signalling from the nerve terminal to the postsynaptic membrane, mediated by agrin and MuSK, is necessary for homeostasis of the adult neuromuscular junction, and that patient anti-MuSK antibodies disrupt this essential signalling pathway, leading to disassembly of pre- and post-synaptic elements of the synapse.

POS-MON-080

TONIC INHIBITION IN CEREBELLAR PURKINJE CELLS OF THE DYSTROPHIN-DEFICIENT mdx MOUSE

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Duchenne muscular dystrophy (DMD) is the second most common genetic disorder in human, affecting 1:3500 male births. It results from mutation in the dystrophin gene causing the absence of the normal full length dystrophin product. Apart from muscle wasting, many case reports have also noted an accompanying mild cognitive impairment in DMD patients. Many of the boys also suffer from sleep disorder. **Purpose:** Here, we investigate the effect of THIP, a hypnotic and antinociceptive that selectively blocks extracellular $GABA_A$ receptors, and SR95531, that in low dose selectively blocks $GABA_A$ phasic currents. **Methods:** Whole-cell recordings of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were performed in cerebellar slices from *mdx* (n=3) and littermate control mice (n=4). All recordings were performed with TTX (0.4μM) in the bathing solution (composition in mM: NaCl 124, KCl 3.2, $CaCl_2$ 2.5, $MgCl_2$ 1.3, $NaHCO_3$ 26, NaH_2PO_4 1.25 and D-glucose 25; bubbled with 95% O_2 and 5% CO_2). Data were analyzed using MiniAnalysis (Synaptosoft™) and Clampfit 9.0 (Axon Instrument Inc). **Results:** We found a significant difference in THIP mediated tonic current in *mdx* mice (76.62 ± 2.572 nA, n=3) compared to littermate controls (44.82 ± 3.821 nA, n=4) (two-tailed t test, p = 0.001). There was no significant difference in the percentage change in amplitude or frequency between *mdx* and littermate controls in the count-matched comparison of average peak mIPSCs recorded before and after 200nM of SR95531. **Conclusion:** Our results indicate a possible increase in extrasynaptic $GABA_A$ receptors in the cerebellar Purkinje cell of dystrophin-deficient *mdx* mice. Dystrophin plays an important role in ion channel localization and stabilization at the post-synaptic membrane.

POS-MON-081

ENVIRONMENTAL ENRICHMENT AMELIORATES THE BEHAVIOURAL PHENOTYPE IN A MECP2-NULL MOUSE MODEL OF RETT SYNDROME

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Rett syndrome (RTT) is characterised by an apparently normal early postnatal development followed by deterioration of acquired cognitive and motor coordination skills. Around 95% of classic RTT cases are caused by mutations in the X-linked gene for methyl CpG-binding protein 2 (MeCP2), a methylation-related transcriptional repressor. **Purpose:** We speculate that environmental modifiers influence RTT outcome and tested environmental enrichment (EE) as a possible treatment in the *MeCP2*^{tm1tam} mouse knockout line. **Methods:** Using age-matched littermates, heterozygous (HET) female and hemizygous (HEMI) male mutant mice were evaluated for amelioration of symptoms after EE from 4 weeks of age. Tests conducted were: rotorod, locomotor activity cell, Y-maze, elevated plus maze, light/dark box, novelty suppressed feeding, forced swim test, and tail suspension test. **Results:** Enrichment improved motor coordination in HET females but not HEMI males. Standard housed (SH) HET mice (n=9) had a coordination deficit on initial exposure to the rotorod ($p<0.05$). The SH HET mice did improve performance with training, but this coordination was lost in subsequent weeks. Enrichment rescued this deficit (n=8) ($p<0.05$). However, it did not differentially affect the performance of HET or HEMI mutants in other behavioural tests. **Conclusion:** Enrichment resulted in a significant rescue of the critical motor coordination deficit in HET *MeCP2*^{tm1tam} mice to WT levels. We are currently investigating whether enrichment-induced changes in levels of BDNF and other proteins correlate with the motor rescue in females.

POS-MON-082

MULTIPLE ADENYLYL CYCLASE ISOFORMS IN CATECHOLAMINERGIC CELL GROUPS CONTROLLING BLOOD PRESSURE

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Purpose: Adenylyl cyclase (AC) is a major second messenger linking G-protein coupled receptors with their effector protein kinase A by controlling the level of cyclic AMP in the neuron. There are nine transmembrane (AC1-9) and one soluble (sAC) isoform of AC, each with unique regulatory properties. We sought to determine whether catecholaminergic neurons important in the control of blood pressure (BP) can be differentiated by AC isoform content. **Method:** Male Sprague Dawley rats were anaesthetized with sodium pentobarbitone, (70 mg/kg ip) and perfused with 4% paraformaldehyde in 0.1MPB. Brainstems were sectioned at 40µm and standard in situ hybridization using isoform-specific probes and immunohistochemistry techniques were performed to determine the distribution of AC mRNA (for AC1,2,4,5,6,7,8, sAC) in the A1 noradrenergic; the rostral C1 adrenergic and the A5 noradrenergic, cell groups, identified by their immunoreactivity to tyrosine hydroxylase (TH-ir). **Results:** All cell groups expressed mRNA for all ACs examined (n=3, for each). Only AC8 was significantly different in its percentage colocalisation (25-30%) with TH-ir neurons compared to all other ACs (60-90%). Most isoforms in all cell groups had over 70% expression in TH-ir neurons. There was no significant difference in the expression of any AC isoform between cell groups ($p<0.01$). **Conclusion:** Similar distributions of AC isoforms are found within each catecholaminergic cell group involved in BP control. The overlapping percentage colocalisation between ACs mean that single catecholaminergic neurons contain multiple isoforms of AC. Different functional cell populations within each catecholaminergic cell group may express unique combinations of AC isoforms. Also, some populations within each cell may contain all isoforms examined.

POS-MON-083

INTRACELLULAR SIGNALING PATHWAYS IN CATECHOLAMINERGIC CELLS ACTIVATED BY GLUCOPRIVATION

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Purpose: Catecholaminergic cells are critical in mediating the body's response to hypoglycaemia. We aimed to determine the intracellular signaling pathways activated in these cells. **Methods:** Adult male Sprague Dawley rats (n=16) were injected intraperitoneally with either 2-deoxyglucose (2DG; 500mg/kg; a glucoprivic agent) or saline. 20-30 minutes later, 10 rats were perfused with 4% paraformaldehyde and brainstem sections processed immunohistochemically for pSer31TH, pSer19TH, pSer40TH (tyrosine hydroxylase-TH-phosphorylated at serine 31, 19 and 40) and pERK1/2. From remaining 6 animals, adrenal glands were processed for western blot analysis of pSer31TH, pSer19TH, pSer40TH.**Results:** pSer31TH was absent in control animals in C1 neurons. 2DG evoked pSer31TH-immunoreactivity in ~50% of TH neurons in rostral C1. pERK1/2 was detected in all neurons expressing pSer31TH. pSer40TH-immunoreactive neurons were significantly reduced ($P< 0.01$) compared to saline controls in brainstem catecholaminergic groups following 2DG (rostral C1: 67±12 vs 21±12; caudal C1: 113±11 vs 60±30 and A1: 125±3 vs 68±16) whereas pSer19TH-immunoreactive neuronal numbers did not change. In the adrenal gland, levels of pSer19TH were similar, pSer31TH was increased 1.8 fold by 2DG but this was not significant, while pSer40TH showed a >2 fold increase ($p<0.01$).**Conclusion:** Glucoprivation results in the activation of catecholamine synthesis in a subpopulation of the C1 cell group via pSer31TH, a process known to require pERK1/2. The down regulation of pSer40TH may represent inhibition of catecholamine signalling in a subpopulation of C1 presympathetic neurons. Phosphorylation of both serine 31 and serine 40 but not 19 in the adrenal gland may be required for adrenaline release following glucoprivation.

POS-MON-084

DISTRIBUTION OF IP3R ISOFORM MRNAS IN BRAINSTEM AND MIDBRAIN CATECHOLAMINERGIC REGIONS OF RATS

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Background: Whilst calcium is essential for neuronal function, little is known about the distribution or function of calcium channel subtypes in specific brain regions including those involved in cardiorespiratory function. Furthermore, no studies to date have investigated inositol trisphosphate receptor (IP3R) isoform specific mRNA expression at the cellular level. **Purpose:** To identify and compare the distribution of three IP3R (1-3) isoforms in catecholaminergic cell groups (A1-A10, C1-C3) located in the brainstem and midbrain tegmentum in rats. **Methods:** Male Sprague Dawley rats (n=9) were transcardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer. Serial coronal sections of the brainstem and midbrain were processed for *in situ* hybridization for an IP3R isoform and immunohistochemistry for tyrosine hydroxylase (TH). Sections were viewed with brightfield and fluorescent microscopy. Cell counts were made. **Results:** The main findings of this study are that IP3R1 and IP3R2 mRNA are absent in all brainstem catecholaminergic cells groups (A1-A6, C1-C3). IP3R1 was densely labeled in various nuclei including cerebellar purkinje neurons and the dorsal cochlear nucleus. Interestingly, a large subset of the dopaminergic A8 (29±3%), A9 (31±5% to 49±2% depending on rostrocaudal level), and A10 cell groups (26±0.4% to 31±5%% depending on rostrocaudal level) also contain mRNA for IP3R1, but not IP3R2. Data for IP3R3 is yet to be analysed **Conclusion:** We have identified the distribution of the three different isoforms of IP3R in rat brainstem including catecholamine cells groups. A subset of dopaminergic neurons in the midbrain that are implicated in the pathogenesis of Parkinson's disease express the transcriptional machinery for IP3R1 but not IP3R2.

POS-MON-085

REGULATION OF THE CENTRAL AMYGDALA CIRCUITRY BY THE KAPPA OPIOID RECEPTOR-DYNORPHIN SYSTEM

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PURPOSE. The endogenous kappa-opioid receptor (KOPR) agonist, dynorphin, is widely expressed by neurons in the central amygdala (CEA) and associated brainstem regions that function in nociception and pain modulation. This study investigated if KOPR signalling limits Fos activation in the rat CeA when opioid-induced hyperalgesia was induced by acute opioid abstinence. Fos mapping was also used to examine if dynorphin neurons respond to a potentially noxious physiological stimulus. METHODS: Fos mapping was performed on 1) groups of 4 rats that experienced systemic morphine (M/S group), naloxone (S/N) or acute abstinence (M/N) after blocking KOPRs with norbinaltorphimine; and 2) groups of 4-6 rats in which cystometry was used to elicit micturition reflexes in awake rats with or without bladder inflammation induced by cyclophosphamide (CYP). RESULTS. In comparison to our previous study, norbinaltorphimine treatment increased Fos neurons counted in the lateral CeA in the S/N and M/N groups, and reduced Fos neurons counted in the medial CeA in the M/N group. There was no change in Fos induced in the capsular CeA. In experiment 2, chronic CYP treatment had no effect on Fos neurons in the CeA, or the parabrachial nucleus which conveys noxious sensory information to the CeA. Nor was any change detected after cystometry in control and CYP rats. However, in the ventrolateral periaqueductal gray which is downstream of the CeA in the descending pain pathway, numerous dynorphin neurons expressed Fos after cystometry, and these were decreased in CYP rats. CONCLUSION. This study has obtained evidence supporting a function of KOPR-dynorphin signalling function under conditions of drug-induced hyperalgesia and noxious visceral stimulation.

POS-MON-086

AUGMENTED RESPIRATORY-SYMPATHETIC COUPLING IN NEONATAL AND JUVENILE SPONTANEOUSLY HYPERTENSIVE RATS

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Purpose. Sympathetic nerve activity (SNA) is altered in mature spontaneously hypertensive (SH) rats compared to their normotensive Wistar-Kyoto (WKY) controls. However, it is unclear whether the altered SNA is a cause or a consequence of the hypertension. We tested the hypothesis that SNA is elevated in pre-hypertensive neonate and juvenile SH rats, and that this may be due to altered respiratory-sympathetic coupling. **Methods**. Perfusion pressure (PP), phrenic nerve activity and thoracic (T8) SNA were recorded simultaneously in the working heart brainstem preparation of male SH and WKY rats at three ages; neonates (postnatal day 9-14), 3-week-old and 5-week-old (n=5 for each group). **Results**. At comparable flow rates, PP was higher in SH rats at all ages. The mean level of SNA was higher in SH rats in the neonates. However the size of respiratory related bursts of SNA were significantly greater in SH rats at all ages ($p<0.05$). Phrenic-triggered averaging of SNA revealed a shift in the peak of respiratory-sympathetic coupling from the inspiratory to the post-inspiratory period with increasing age in SH rats only. Respiratory related oscillations in PP, Traube-Herring (TH) waves, were significantly larger in SH rats than WKYs at all ages (neonates 0.6±0.4 vs. 1.8±0.4mmHg; 3-week-old 2.8±0.7 vs. 5.6±1.5mmHg; 5-week-old 1.5±0.8 vs. 9.8±1.5mmHg, $p<0.05$). **Conclusions.** Increased SNA occurs in SH rats in early postnatal periods and shows enhanced respiratory coupling with age. This is reflected in altered arterial tone, even in the "pre-hypertensive" phase, with elevated PP and TH waves. We speculate that these increased respiratory-related bursts of SNA might, over time, be a causal factor in the development of hypertension.

POS-MON-087

MICROINJECTION OF PACAP-38 INTO THE ROSTRAL VENTROLATERAL MEDULLA OF SPRAGUE-DAWLEY RATS CAUSES SYMPATHOEXCITATION PARTIALLY MEDIATED THROUGH THE PAC1/VPAC2 RECEPTORS

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Purpose: To investigate the effects that microinjection of PACAP-38 into the rostral ventrolateral medulla has on heart rate, mean arterial pressure (MAP) and splanchnic sympathetic nerve activity. **Methods:** Adult male Sprague-Dawley rats (n=17) anaesthetized with urethane (1.5g/kg; ip) were ventilated and paralysed. Splanchnic nerve activity was recorded as a measure of sympathetic nerve activity, heart rate was derived from an ECG and the carotid artery was cannulated for blood pressure measurement. An occipital craniotomy was performed for the microinjection of vehicle and PACAP-38 (n=7) or PACAP(6-38) followed by PACAP-38 (n=5) or PACAP(6-38) only (n=5) into the rostral ventrolateral medulla. **Results:** PACAP-38 significantly increased MAP by a maximum of 35 ± 5mmHg ($P < 0.0001$), splanchnic sympathetic nerve activity by 93 ± 9% ($P < 0.0001$) and heart rate by a maximum of 36 ± 4bpm ($P < 0.0001$). While sympathetic nerve activity and heart rate remained elevated over the 2h period, MAP returned to baseline 1h after PACAP-38 administration. The PACAP-38 heart rate, MAP and splanchnic sympathetic nerve responses were all significantly attenuated ($P < 0.001$) but not abolished by pre-treatment with the antagonist PACAP(6-38). PACAP(6-38) had initial PACAP-like effects on MAP, heart rate and splanchnic sympathetic nerve activity but the overall responses were no different to the post-antagonist PACAP effects. **Conclusion:** PACAP-38 in the rostral ventrolateral medulla elicits long-lasting splanchnic sympathoexcitation and tachycardia as well as a recoverable pressor effect. These responses are partially mediated through the PAC1 and VPAC2 receptors. The PACAP antagonist PACAP(6-38), whilst having initial agonist-like effects at the dose used in this study, is an effective antagonist.

POS-MON-088

THE EFFECTS OF GALANIN MICROINJECTION IN THE VENTROLATERAL MEDULLA ON THE CARDIOVASCULAR AND RESPIRATORY RESPONSE TO HYPERCAPNIA AND HYPOXIA

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Purpose: To investigate the effects of microinjecting galanin into discrete nuclei in the ventrolateral medulla that are crucial for the maintenance of blood pressure and respiration, on the cardiorespiratory responses to hypercapnia and hypoxia. **Methods:** Bilateral microinjections of galanin (1mM, 50nL) were made into the rostral ventrolateral medulla (RVLM), Bötzinger complex and pre-Bötzinger complex in urethane anaesthetised, mechanically ventilated, vagotomised Sprague Dawley rats (N=24) whilst recording splanchnic sympathetic nerve and phrenic nerve discharge. Following microinjections of galanin, the cardiorespiratory response to hyperoxic hypercapnia (10% CO_2 in O_2) and normocapnic hypoxia (10% O_2 in N_2) were recorded. **Results:** Microinjections of galanin into the rostral ventrolateral medulla (RVLM) caused a reduction in baseline sympathetic discharge (-35.6 ± 4.7% of baseline; N=7) and blood pressure (-17.5 ± 2.5mmHg; N=7) and attenuated the pressor response caused by hypercapnia (from 27.2 ± 1.9mmHg to 6.5 ± 1.3 mmHg; N=5). Microinjections of galanin into the Bötzinger complex and Pre-Bötzinger complex of the ventral respiratory column evoked a dys-rhythmic breathing pattern. Microinjections in the Bötzinger complex (N=6), but not the Pre-Bötzinger complex (N=6), increased the peak rate response, but not the amplitude response, of phrenic nerve discharge to both hypercapnia (from 54.7 ± 1.6 breaths/min to 64.2 ± 6.0 breaths/min) and hypoxia (67.7 ± 5.2 breaths/min to 82.0 ± 8.9 breaths/min). **Conclusion:** Galanin appears to modulate the sensitivity of respiratory rhythm generating neurons to changes in blood gases. Furthermore, galanin reduces the activity neurons in the RVLM important for tonic vasomotor sympathetic drive and the sympathoexcitatory effects of blood acidosis.

POS-MON-089

POTENTIATION OF THE HYPERTENSIVE EFFECT INDUCED BY INTRACEREBROVENTRICULAR INJECTION OF TACHYKININ NK-3 AGONIST SENKTIDE FOLLOWING HIGH THORACIC SPINAL CORD INJURY

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People with high level spinal cord injury (SCI) suffer from persistent hypotension due to loss of tonic input to sympathetic preganglionic neurons from vasomotor centers in the brain stem. Neuroendocrine response such as increased release of vasopressin is believed to participate in compensatory mechanisms to maintain mean arterial pressure (MAP) but few reports have addressed the central mechanism. Vasopressin release is modulated by central tachykinin NK-3 receptors. Indeed, previous reports have shown that intracerebroventricular (i.c.v) injection of the NK-3 receptor agonist senktide leads to increase in MAP and heart rate (HR) through the release of vasopressin and the stimulation of the sympathetic nervous system, respectively. Purpose: Test the hypothesis that the hypertensive effect of i.c.v senktide is amplified following a high thoracic SCI. Methods: Rats were chronically implanted with a guide cannula into the right lateral ventricle. For MAP and HR recording, rats were implanted with data sciences radio-telemetric probes. Senktide (650 pmol, n=9) was injected in freely moving male rats before and two weeks after a complete spinal cord transection at thoracic level 4 (T4). Results: Two weeks after T4 SCI, the rise in MAP induced by senktide was significantly increased in magnitude ($P < 0.001$). This effect was accompanied by a biphasic response in HR characterised by an initial long lasting bradycardia followed by a tachycardia. Conclusion: Although the mechanisms remain to be elucidated, our results suggest that the release of vasopressin induced by senktide is potentiated in T4 transected rats. NK-3 receptors might contribute to the maintenance of MAP following high thoracic SCI.

POS-MON-090

8-OH-DPAT ATTENUATES CARDIOVASCULAR RESPONSES TO CONDITIONED FEAR AND RESTRAINT STRESS

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Between 48 and 82 % of myocardial infarctions are preceded by environmental triggers, of which emotional upset is the most common. Theoretically, drugs which could block the effects of emotional upset on cardiovascular function could be used to prevent that triggering. **Purpose**. We sought to test the effect of 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist, on cardiovascular changes during two types of emotional stress in the rat. **Methods**. Eight animals were implanted with telemetric probes for measurement of heart rate (HR), mean arterial pressure (MAP) and activity, and later fear conditioned to context. They were then tested for conditioned fear to context and restraint stress, 30 min after i.p. injections of either saline, 0.05 or 0.25 mg/kg 8-OH-DPAT, in a counterbalanced order. **Results**. Administration of 0.25 mg/kg 8-OH-DPAT significantly ($p < 0.05$) reduced HR and MAP responses to restraint (54 ± 15 and 48 ± 14 % decrease, respectively), and HR and MAP responses to fear (41 ± 9 and 77 ± 8 % decrease, respectively). Administration of 0.05 mg/kg 8-OH-DPAT had a significant effect on the restraint HR, but not on other responses. Baseline HR and MAP were not altered by the drug. **Conclusion**. 8-OH-DPAT given systemically can reduce cardiovascular responses to emotional stress without altering basal HR and MAP. This opens the possibility that 5-HT_{1A} agonists, like buspirone, could be used clinically as an alternative to prevent the cardiovascular effects of emotional stress.

POS-MON-091

ETHANOL ENHANCES THE RESPIRATORY ACTION OF THE TRPV1 AGONIST, RESINIFERATOXIN, IN THE NUCLEUS OF THE SOLITARY TRACT

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The transient receptor potential vanilloid 1 (TRPV1) channel possesses multiple binding sites including those for protons, ethanol (EtOH) and the vanilloids, capsaicin and resinferatoxin (RTX). **Purpose:** To determine the effects of EtOH on the respiratory response to microinjection of RTX into the commissural nucleus of the solitary tract (cNTS). **Methods:** Hooded Wistar rats (10 weeks) were anaesthetised (urethane 0.5 g/kg ip, 0.5 g/kg sc), mounted in a stereotaxic apparatus and brainstem exposed. Respiratory movements were recorded using impedence electrodes and converted to frequency tidal volume (VT) and minute ventilation (VE). **Results:** Resting (pre-injection) VE ranged between 3.04 and 4.3 ml/kg. Baseline frequencies (breaths/min) prior to microinjection of the EtOH/saline vehicle (97 ± 3, n = 15 injections) and RTX (106 ± 3, n = 19 injections) were similar. Microinjection of 10, 20 and 35% EtOH in normal saline (500 nl) into the cNTS decreased frequency (-13 ± 11, -30 ± 8 and -8 ± 2, respectively; n = 3-7) but not VT. Microinjection of RTX (50 pmol) in 10% EtOH was followed by a small decrease in frequency (-5 ± 22, n = 5) but had a negligible effect on VT. However, the maximum respiratory response (bradypnoea) evoked by RTX was potentiated in a concentration-dependent way by 20% (-24 ± 10, n = 9) and 35% EtOH (-67 ± 8, n =4). **Conclusion:** The present study suggests that EtOH improves penetration of RTX into sensory terminals in the cNTS and/or that EtOH interacts directly with TRPV1 to enhance the actions of RTX.

POS-MON-092

DO THE DESCENDING PATHWAYS MEDIATING CARDIORESPIRATORY RESPONSE FROM THE DMH RELAY IN THE MIDBRAIN PAG?

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Purpose: The dorsomedial hypothalamus (DMH), is an essential brain region mediating the physiological response to stress. The cardiovascular components of the response include increases in blood pressure, heart rate (HR) and sympathetic nerve activity. In addition, the cardiovascular changes are also associated with increases in respiratory activity. The sympathetic descending pathways from the DMH include synapses in the rostral ventrolateral medulla and the medullary raphe, but it is not clear whether these projections from the DMH to the medulla are direct or indirect. It has been suggested recently that the sympathoexcitatory pathway from the DMH includes a synapse in the dorsolateral periaqueductal grey (PAG) in the midbrain. We tested the hypothesis that the descending pathways mediating sympathoexcitatory and respiratory responses from the DMH include an essential synapse in the PAG. **Methods**: Arterial pressure, HR, renal sympathetic nerve activity (RSNA), and phrenic nerve activity (PNA) was recorded in urethane anaesthetized rats. **Results**: Microinjection of bicuculline in the DMH caused increases in mean arterial pressure (MAP), HR, RSNA and PNA burst rate. Multiple injections of muscimol in the dorsal/dorsolateral part of the PAG (n=9) did not significantly attenuate the increases in MAP, HR, RSNA and PNA. Similarly, multiple microinjections of the local anaesthetic lignocaine (n=4), which blocks both neurons and fibers of passage, also did not attenuate the cardiorespiratory responses to the DMH activation. **Conclusion**: The results indicate that the cardiorespiratory response evoked from the DMH is not dependent on synapses in the midbrain PAG, and thus is mediated either by direct projections from the DMH to medullary nuclei, or via supramedullary nuclei outside the PAG.

POS-MON-093

EFFECT OF HCN CHANNEL BLOCKERS ON VAGAL CONTROL OF AIRWAY SMOOTH MUSCLE TONE IN GUINEA PIGS

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Hyperpolarization activated cyclic nucleotide gated (HCN) channels help regulate neuronal membrane potential. The aim of this study was to determine whether HCN channel blockers (ZD7288 or Cs+) alter vagal control of airway smooth muscle. In urethane anaesthetized, artificially ventilated guinea pigs (pretreated with propranolol (2mg/kg) and CP99994/ SR48968 (0.3mg/kg) to prevent sympathetic and neurokinin mediated responses) bilateral electrical stimulation (32Hz, 10 sec trains) of the vagi produced voltage dependent (1-12V), atropine sensitive increases in pulmonary inflation pressure. Treatment with ZD7288 (2mg/kg, iv, n=7), but not vehicle (n=6) potentiated bronchoconstriction (voltage producing 50% maximum contraction = 6.0 ± 0.7 and 3.3 ± 0.7 in vehicle and ZD7288 treated animals, P<0.05). Vagus nerve-evoked contractions in vitro were also potentiated by 5mM Cs+ (n=8). Thus, at stimulus intensities of 10.3 ± 1.3 volts, tracheal contractions averaged 25.4 ± 4.5 and 62.7 ± 12.5 percent of the maximum before and after bath application of Cs+, respectively. Furthermore, Cs+ treated preparations began exhibiting spontaneous rhythmical contractions (onset = 17.0 ± 4.9 minutes post Cs+, peak magnitude = 29.6 ± 8.2 percent of the maximal attainable tracheal contraction). Spontaneous contractions were (a) abolished by 1μM atropine (n=3) and 1μM tetrodotoxin (n=3), confirming their cholinergic and neuronal nature, (b) reversed by the ganglionic blocker hexamethonium (100mM, n=4) and (c) almost absent in tissues studied following 48 hours in organotypic culture. Preliminary immunohistochemical experiments (n=2) show that preganglionic neurons in the nucleus ambiguus may express HCN1 and HCN4 channel subtypes. These data suggest that inhibition of HCN channels on vagal preganglionic neurons projecting to the airways may increase vagal drive to the airway smooth muscle.

POS-MON-094

CARDIOVASCULAR EFFECTS OF ACTIVATION OF GHRELIN RECEPTORS IN THE SPINAL CORD OF THE RAT

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The peptide hormone ghrelin is best known for its role in growth hormone release and control of appetite. Recently, ghrelin has been shown to have growth hormone independent cardiovascular actions, in both human and animal. We have recently discovered that peripheral administration of a ghrelin receptor agonist that enters the central nervous system elevates blood pressure (BP). **Purpose**: The studies were designed to determine the mechanisms by which activation of ghrelin receptors in the spinal cord stimulate sympathetic vasoconstrictor pathways, and whether cardio-accelerator pathways are also activated. **Methods**: Experiments were conducted on 106 Sprague-Dawley rats anaesthetized with ketamine and α-chloralose. **Results**: Intravenous bolus (0.5-10mg/kg) of ghrelin agonists CP464709 or GSK894490 elicited an initial rapid hypotension of 5-30%, followed by a sustained hypertension of 15-30%. No changes in heart rate were seen. The hypotensive response was blocked by hexamethonium and reduced to 7% by phentolamine. Direct application of either non-peptide agonist into the intrathecal space from thoracic 9 (T9) to lumbar 4 (L4), increased BP at all sites, but most significantly by 100±25% at T9 and 73±1% at T10. Ghrelin, but not des-acyl ghrelin also increased BP. No cardio-accelerator effect was revealed when BP was blocked by phentolamine, although isoprenaline caused a substantial heart rate increase of 41±14%. No changes in ECG were observed. **Conclusion**: Activation of ghrelin receptors in the spinal cord stimulates vasoconstrictor, but not cardio-accelerator pathways. Activation of peripheral receptors causes vasodilation, but not cardiac effects.

POS-MON-095

BLOCKADE OF 5-HT2A RECEPTORS SUPPRESSES HYPERTHERMIC RESPONSES ELICITED BY SOCIAL DEFEAT IN RATS

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Purpose: To determine whether 5-HT2A receptors are involved in mediating autonomic responses to psychological stress. **Methods:** in Hooded Wistar rats (n=15) instrumented with radiotelemetric transmitters we assessed changes in the heart rate, arterial pressure and core body temperature elicited during social defeat. Fifteen min prior to entering the resident's cage, intruders received s.c. injection of either selective 5-HT2A receptor antagonist SR-46379B (0.3, 1.0 or 3.0 mg/kg) or vehicle. **Results:** After vehicle, social defeat induced substantial hyperthermic (+1.1±0.1°C), tachycardic (+142±11 bpm) and pressor (+22±3 mmHg) responses. SR46349B at doses 0.3 and 1.0 mg/kg abolished hyperthermia, and at the dose of 3.0 mg kg reverted it to the hyporthermia (-0.41±0.1°C; n=7, p<0.01 for all doses). The drug reduced tachycardia (to +115±13 bpm, n=15, p<0.05) only when applied at the highest dose, and did not affect pressor response at any dose (n=8). Anti-hyperthermic effect was still well expressed 1.5 h after injection, whereas anti-tachycardic effect waned at the end of defeat (30 min after injection). **Conclusions:** i) activation of 5-HT2A receptors is essential for the expression of stress-induced hyperthermia; ii) contribution of these receptors to the stress-induced tachycardia is minor; iii) 5-HT2A receptors do not mediate rise in arterial pressure during psychological stress.

POS-MON-096

SPINAL SOMATO-SYMPATHETIC REFLEXS IN HUMAN SPINAL CORD INJURY

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Purpose: Spinal Cord Injury (SCI) can cause partial or complete loss of central control of sympathetic function below the lesion. As segmental circuitry is intact, visceral or somatic stimuli originating below lesion can cause a reflex activation of sympathetic vasoconstrictor neurones. The purpose of the present study was to assess the input-output relationship of the somato-sympathetic spinal reflex in SCI, to determine if the reflex response is dependent upon the degree of sensory input. **Methods:** Continuous blood pressure, ECG, respiration and changes in cutaneous blood volume (photoelectric plethysmography) in the fingers and toes were recorded in 11 subjects with SCI (C4-T7). Electrical stimuli (1ms, 1.8-10 mA) of varying duration and magnitude were applied via surface electrodes to the abdominal wall (below lesion): 20 Hz for 1s; 1 Hz for 20s; 20 Hz for 20s and 20 Hz for 1s alternating on and off for 20s. **Results:** Six out of eleven SCI subjects had blood pressure and cutaneous vasoconstrictor responses to the electrical stimulation. On average, the responses to the prolonged 20 Hz, 20s stimulus train were similar in both duration and magnitude to the brief 20 Hz, 1s stimulus. **Conclusion:** These observations demonstrate that the sympathetically mediated vasoconstrictor responses to somatic afferent stimulation may not be directly related to the number of pulses or to the duration of the stimulus train. The recruitment of cutaneous vasoconstrictor neurones may be limited to the initial afferent barrage evoked by a stimulus train. Whether this is due to habituation of the spinal somato-sympathetic reflex or neuromuscular fatigue of the blood vessels remains to be determined.

POS-MON-097

CHRONIC HEART FAILURE INDUCES CHANGES IN AUTONOMIC NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

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Pre-autonomic and neuroendocrine neurons in the hypothalamic paraventricular nucleus (PVN) play important roles in regulating blood pressure and fluid balance. Anatomical and physiological evidence suggests that, in chronic heart failure (CHF), changes occur in the anatomy and physiology of the PVN to compensate for the heart's reduced ability to pump blood. In this study, we investigated the effect of CHF on hypothalamic neurons immunoreactive for corticotropin releasing factor (CRF), vasopressin (VP), nitric oxide synthase (NOS), enkephalin (ENK) and pro-ENK. We ligated the left coronary arteries of male rats to induce myocardial infarction, which leads to the development of CHF when 40% or more of the left ventricle is affected. The arteries of sham-operated control rats were not ligated. Rats were perfused six to eight weeks after surgery. Sections of perfused hypothalamus from control and CHF rats were stained with peroxidase immunohistochemistry. Immunoreactive neurons were counted in the PVN (CRF, VP, NOS) and anterior hypothalamic area (ENK, pro-ENK). We found that CRF- and VP-immunoreactive neurons were more numerous in the PVN of rats with infarcts occupying 40% or more of their left ventricles than in control rats ($p<0.05$, n=5-6 rats/group). In rats with CHF, there was a trend towards a decrease in the numbers of NOS-immunoreactive neurons in the PVN ($p=0.07$, n=5/group). ENK neurons were not found in the PVN itself, as previously reported; and the number of ENK- ($p=0.34$, n=4/group) and pro-ENK-immunoreactive ($p=0.57$, n=3/group) neurons did not differ between CHF and control rats. These results suggest that CHF may lead to widespread changes in the neurochemistry of hypothalamic neurons involved in the regulation of autonomic function.

POS-MON-098

THE CONTRIBUTION OF NECK AFFERENTS TO CARDIOVASCULAR FUNCTION IN SITTING HUMANS

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Purpose: The influence of the large afferents from the neck muscle on autonomic and cardiovascular function was examined. Our previous experiment suggested that a vibratory stimulus applied to the right dorsal neck influenced cardiovascular function. However, factors such as arousal effect and the laterality of the response which may confound interpretation remain to be determined. **Methods:** Further investigations used a vibratory stimulus, applied to the left dorsal neck and right anterior tibialis in sitting humans (n = 10) while ECG, blood pressure, finger blood flow, and forearm blood flow were non-invasively measured. Two-way repeated measures analysis of variance was used and statistical significance level was set at $p <$ 0.05. **Results:** An illusion of head motion was perceived during neck vibration in most participants (n = 9), but not during leg vibration. Neck vibration significantly decreased forearm blood flow during the stimulation period. Following cessation of neck vibration, mean arterial pressure decreased and finger blood flow increased. On the other hand, leg vibration revealed a significant decrease in heart rate and an increase in finger blood flow during stimulation, while heart rate reduction was seen after cessation of neck vibration. **Conclusion:** Leg vibration induced cardiovascular responses distinct to neck vibration so the effects of neck vibration presumably included an arousal effect. This effect appeared to be superimposed on genuine cardiovascular effects induced by activation of neck afferents. Therefore, this study may indicate that the neck afferents contribute to cardiovascular regulation and this affect seemed not to include laterality.

POS-MON-099

SOMATOSYMPATHETIC REFLEXES EVOKED BY C-NOCICEPTOR ACTIVATION ARE NOT EXPRESSED IN THE CERVICAL SYMPATHETIC NERVE

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Purpose: Inputs from unmyelinated and myelinated nociceptors drive sympathetic responses to painful stimuli. Work in the cat indicates that nerves innervating different target tissues (e.g. skin versus muscle) exhibit qualitatively different somatosympathetic responses. However, no comparable systematic examination of somatosympathetic responses has been made in the rat. Here we compare responses to sciatic nerve (SN) stimulation simultaneously recorded in the cervical and splanchnic sympathetic nerves and medullary cardiovascular neurones. **Methods:** Adult male Sprague Dawley rats (250-450 g) were anaesthetised with urethane (1.3 g kg^{-1} i.p.), cannulated, vagotomised, paralysed, and ventilated. In one group of experiments, the cervical and splanchnic sympathetic nerves were recorded (N=6); in a separate group of rats, extracellular recordings were made from barosensitive neurones in the rostral ventrolateral medulla (RVLM; N=16). The SN was prepared for stimulation in all experiments. **Results:** SN stimulation evoked characteristic biphasic responses in splanchnic nerve activity (N=6), but only monophasic responses, analogous to the first phase of the splanchnic response, in the cervical nerve (N=4). Most RVLM neurones exhibited a biphasic response, although the relative magnitudes of the two phases were highly variable, and in some cases longer latency volleys were completely absent. **Conclusions:** The biphasic splanchnic response is likely due to the arrival of two distinct afferent volleys at the RVLM, whereas cervical sympathetic preganglionic neurones are driven by the low latency component alone. These results suggest that somatic inputs from myelinated and unmyelinated nociceptors are differentially channelled within the somatosympathetic reflex pathway. This assertion is supported by the observation that barosensitive RVLM neurones exhibit variable response profiles.

POS-MON-100

CENTRAL RESPIRATORY PATTERNS ARE MODULATED BY BARORECEPTOR INPUT

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Purpose: The question of whether baroreceptor input modulates respiratory drive remains contentious. The current study was designed to determine the sensitivity of central respiratory drive (CRD) to baroreceptor stimulation. **Methods:** Experiments were conducted on adult male Sprague-Dawley rats (250-350 g; N=7), deeply anaesthetised with urethane (1.3g kg-1 i.p.). Rats were cannulated, intubated and vagotomised. The phrenic and splanchnic sympathetic nerves were prepared for recording. 1-1.5 cm of aortic depressor nerve (ADN) was isolated between the carotid bifurcation and brachial plexus and prepared for stimulation. All nerves were embedded in silicone rubber. The effects of tetanic and intermittent ADN stimulation at different phases of the respiratory cycle on phrenic nerve activity were assessed and compared to those evoked by intravenous phenylephrine (PE) and angiotensin II (Ang II). Artificial ventilation rates were varied to increase or reduce CRD. **Results:** Intravenous phenylephrine caused a large increase in blood pressure that caused an increase in CRD frequency. This effect was not observed when equivalent pressor effects were evoked by Ang II. Tetanic ADN stimulation caused a significant lengthening of expiration, with no effect on inspiratory duration, regardless of what point in the respiratory cycle the ADN was stimulated. Intermittent ADN stimulation increased the coefficient of variability of the respiratory period without significantly affecting mean expiratory time. This effect was most profound when respiratory drive was lowered by increasing the frequency of ventilation. Waveform averages of phrenic discharge were not affected by ADN stimulation. **Conclusions:** Our results indicate that baroreceptor stimulation is capable of modulating the respiratory rhythm in a non-linear fashion. The qualitative effects evoked by ADN stimulation depend on the modality of stimulation.

POS-MON-101

MANY INSPIRATORY AUGMENTING PREMOTONEURONS IN THE RAT ROSTRAL VENTRAL RESPIRATORY GROUP EXPRESS MUSCARINIC M2 RECEPTOR

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Background: Cholinergic transmission modulates respiratory motor output from the brainstem. Nevertheless, the expression of muscarinic receptor subtype(s) on bulbospinal respiratory premotor neurons is not known. **Purpose:** To investigate the distribution of muscarinic 2 receptor (M2R) mRNA in the medullary rostral ventral respiratory group (rVRG). **Methods:** In separate studies, *in situ* hybridisation for M2R mRNA was combined with immunohistochemistry for (i) retrogradely (cholera toxin B, CTB) traced respiratory premotoneurons in the upper thoracic spinal cord (ii) neurobiotin-filled inspiratory augmenting (IAUG) neurons in the rVRG (iii) markers of different neuronal types in the ventrolateral medulla, including tyrosine hydroxylase (TH), neurokinin 1 receptor (NK1R), glycine transporter 2 (GLYT2), and high affinity choline transporter (hHCT). **Results:** (i) Densely labelled M2R mRNA containing neurons (M2R+) are apparent in the rVRG region. 53±1% of bulbospinal rVRG neurons that drive intercostal and abdominal motoneurons were M2R+ (134±5 out of 261±19 cells, n=3). (ii) 66% (2/3) of IAUG neurons in the rVRG were M2R+. (iii) M2R+ neurons were also colocalised with NK1R-ir, but never TH-ir, and apposed by GLYT2-ir terminals at the level of the rVRG. Few M2R+ vagal premotoneurons (defined by hHCT-ir) were intermingled with M2R+ VRG neurons, especially at more caudal levels. **Conclusion:** In the rat, central inputs to intercostal and abdominal muscles originate in largely cholinoceptive neurons in the VRG. M2R mRNA is expressed by a large subset of bulbospinal neurons in the VRG. Part of this large subset of neurons also contain NK1R, receive inhibitory inputs from glycinergic neurons and is IAUG in nature.

POS-MON-102

INVESTIGATING THE BIOPHYSICAL AND NEUROTOXIC PROPERTIES OF PrP PEPTIDES MODELLED ON C-TERMINAL FRAGMENTS GENERATED FROM ENDOGENOUS CLEAVAGE

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Purpose: Cellular prion protein (PrP^C) can undergo proteolytic cleavage at around residue 111 (α-cleavage) or around residue 90 (β-cleavage). Increased β-cleavage is generally found in prion diseases, fatal neurodegenerative disorders associated with conformational transformation of PrP^C to a pathogenic isoform, PrP^{Sc}. Various peptide fragments of PrP have been studied, such as PrP106-126, which although neurotoxic, are of uncertain physiological relevance. Alpha cleavage is hypothesized as potentially important for abrogating toxicity in peptides containing the "neurotoxic" segment 106-126. To explore the role of endogenous processing in modulating the potential neurotoxicity of the 106-126 region, we studied HuPrP90-144 (β-cleavage model) and HuPrP111-144 (α-cleavage model). **Methods:** The properties of PrP peptides representing α and β cleavage has been investigated using an established cell viability assay and a number of biophysical techniques. **Results:** CCK8 cell viability assays (n=3) show huPrP90-144 is toxic to cells, whereas huPrP111-144 is not. However, this toxicity is not a simple consequence of increased aggregability (fluorometric studies (n=3) show huPrP111-144 aggregates faster than huPrP90-144), Cu^{2+} binding (EPR studies (n=1) show that both peptides bind Cu^{2+} ions), nor enhanced quenching or production of ROS (not different between the peptides), as determined using the dichlorofluorescein assay (n=4). **Conclusion:** Overall, our results support the view that α-cleavage is protective to the cell. Investigations so far have not revealed the biophysical basis or mechanism of toxicity, but studies to date show this does not simply correlate with aggregability, Cu^{2+} binding or production of ROS.

POS-MON-103

SEIZURES IN THE NEWBORN HYPOXIC-ISCHEMIC BRAIN. SILENT KILLERS?

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Perinatal hypoxia ischemia (HI) is a significant cause of death and neurodevelopmental disability. Loss of oxygen and glucose supply leads to excitotoxic neuronal cell injury and death; over-excitation may also manifest as seizures. The newborn brain is highly susceptible to seizures although it is unclear if seizures exacerbate HI injury. Neonatal seizures are often silent and current methods of EEG monitoring are limited, meaning that many seizures will go undetected. Clinical seizures may disappear with anticonvulsant treatment but remain as silent seizures. Purpose: To investigate the effect of both clinical and sub-clinical seizures on HI injury in the newborn piglet brain. Methods: Hypoxia (n=27) was induced by reducing FiO_2 to 0.04 for 30 min. EEG was monitored to attain low amplitude EEG (<5 μV, LAEEG) and hypotension induced for the final 10 min. Daily EEG amplitude was recorded to determine seizure activity. At 72h post-insult animals were euthanased and neuropathological injury analysed using histology, MAP2 and GLAST1b immunohistochemistry. Results: Silent seizures were recorded in 52% of piglets while clinical seizures were observed in 27%. 22% of piglets showed no seizure activity. Histological injury was significantly greater in both seizure groups compared to HI animals with no seizure activity ($p < 0.05$). MAP2 immunoreactivity was significantly decreased in both seizure groups. GLAST1b was detected in all HI brains; increased immunoreactivity was observed in animals with seizures. Conclusion: Presence of seizures, regardless of type, significantly worsened outcome. Long term EEG monitoring following birth asphyxia is critical to identify and thus treat silent seizures.

POS-MON-104

HYPOXIA/HYPOGLYCEMIA RESISTANCE IN CEREBELLAR PURKINJE CELLS OF THE mdx MOUSE

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Dystrophin, absent or defective in Duchenne muscular dystrophy, is found in certain cells in the brain, including cerebellar Purkinje cells. **Purpose:** To examine the effect of the absence of dystrophin on synaptic transmission in the *mdx* mouse. **Method:** Adult mice (*mdx* and littermate controls) were killed with halothane/ decapitation. Intracellular recordings were made from Purkinje cells (PCs) in parasagittal cerebellar slices (250 μm). Excitatory postsynaptic potentials (EPSP) were evoked by electrically stimulating the parallel fibre synaptic inputs every 30 s. The slice was made hypoxic and hypoglycemic (HH) by using superfusate gassed with 95% N_2 5%CO_2 and containing equimolar sucrose instead of glucode. **Results:** PCs were challenged with and without glucose in the bath, with a 20 min recovery interval. After both challenges the level of recovery of the EPSP amplitude and initial slope were significantly greater ($p=0.03$) in the *mdx* PCs (n=9) compared to controls (n=5). In experiments with prolonged hypoxia, evoked responses in PCs were abolished significantly earlier ($p=0.03$) in the *mdx* (n=10) at 13.9 min compared with controls (n=7) at 20.2 min. Following a hypoxic challenge, the addition of 100 μM glibenclamide, an ATP sensitive K^+ channel blocker, to the bath showed a substantially greater EPSP on recovery than pre-hypoxia levels in the mdx compared to controls. **Conclusion:** In contrast to the reported hypersensitivity of dystrophin positive cells in the hippocampus, cortex and cerebellum, we showed a stronger post-hypoxic recovery of synaptic transmission in the presence of low glucose in *mdx* compared to control PCs suggesting that dystrophin-deficient Purkinje cells may be more resistant to repeated or prolonged periods of hypoxia.

POS-MON-105

ASTROCYTE REMODELLING IN THE HYPOXIC BRAIN: A CONSEQUENCE OF CYTOSKELETAL DEPOLYMERISATION?

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Purpose: Hypoxia is a major cause of brain damage in both young and old humans. The mechanisms by which brain damage occurs are not fully understood. Astrocytes function to maintain the extracellular environment, regulating molecules such as neurotransmitters, glucose, water and ions. Thus, normal astrocyte function is required for normal neuronal function. **Methods:** Neonatal pigs (N=6) were anaesthetised and exposed to 4% oxygen for 30min, and allowed to recover for 72hr. Control animals (N=6) were littermates exposed to anaesthesia, but no hypoxia. Pigs were euthanased, brain tissues removed and 300µm-thick slices were cultured in oxygenated AMES media for 75min in the presence of ligands such as glutamate. **Results:** We have shown that changes in the glial architecture underpin the subsequent damage or loss of neurons in the hypoxic brain. We hypothesised that changes in astrocyte morphology may be initiated by rises in extracellular glutamate, causing phosphorylation of GFAP, depolymerising the GFAP and destabilising the astrocyte cytoskeleton. Preliminary data from brain slices incubated with L-glutamate and the glutamate transporter blocker TBOA revealed a rapid rise in GFAP immunoreactivity, especially in grey matter regions. This observation is compatible with the notion that the polymeric form of GFAP was converted to the more easily detectable monomeric form. **Conclusions:** Depolymerisation of GFAP, caused by rising glutamate levels in the hypoxic brain, may be important in the pathophysiology of hypoxic brain damage. We are currently generating phosphorylation-specific antibodies against GFAP to validate this view. Inhibiting phosphorylation of GFAP may provide a novel therapy for minimising hypoxic brain damage.

POS-MON-106

MRI AND SEIZURE DATA AND ASSOCIATED LEVEL OF INJURY AFTER HYPOXIA-ISCHAEMIA IN THE NEWBORN PIGLET

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Purpose: Perinatal hypoxia can result in neurological injury and the hypoxic event is usually detected after birth. Seizures often occur after severe hypoxia-ischaemia (HI). The aim of this study was to determine if HI piglets that developed seizures exhibited more severe neurological damage compared with HI piglets without seizures. **Methods:** Piglets (<24hr) were anaesthetised, ventilated and catheterised for monitoring and blood sampling. Hypoxia was induced by reducing F_iO_2 to 0.04. EEG was monitored to attain low amplitude EEG (<5µV, LAEEG). Hypoxia continued (30min) and in the final 10min F_iO_2 was altered to induce hypotension. On reoxygenation (F_iO_2=1.0) anaesthesia was ceased. Daily EEG amplitude was recorded. Brain MRIs were performed at 24 and 72h post-hypoxia to obtain ^{1}H-spectroscopy, ADCs and T2-signals. Piglets were euthanased after 72h scan, brains removed and fixed for H&E staining. **Results:** 27 piglets showed histological injury after HI (seizures n=21). EEG amplitude in hypoxic piglets without seizures was higher compared with seizure piglets ($p<0.05$). Day1 MRI showed lower ADCs and higher T2 signals in the brains of seizure piglets ($p<0.05$). Piglets with seizures had significantly worse outcomes assessed through MRI and histology. A predictive injury score was formulated based on day1 MRI, EEG and neurobehavioural performance; regression analysis showed a strong relationship between day1 injury score and histological injury ($r^2=0.818$, $p<0.0001$). **Conclusion:** The presence of seizures (electrical and/or clinical) was associated with a greater degree of histological brain injury. There is a need for early MRI and EEG monitoring after birth asphyxia for better detection and treatment of seizures and prognostication.

POS-MON-107

SHORT AND LONG-TERM LOSSES OF CORTICOTROPIN-RELEASING FACTOR NEURONS ARE EVIDENT AFTER P3 HI IN THE IMMATURE RAT BRAIN

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Purpose: Brain injury resulting from hypoxia-ischemia (HI) in the preterm neonate is associated with numerous neurological impairments including deficits in cognitive, learning and memory functions. Neurons in the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST) and paraventricular nucleus of the hypothalamus (PVN), in particular corticotropin-releasing factor (CRF) neurons, are important contributors to these functions. However it is not known whether preterm HI affects neuronal populations in the CeA, BNST and PVN. **Methods:** We examined the effects of preterm HI by subjecting post-natal day 3 (P3) Sprague Dawley rat pups to HI (ligation of the right common carotid artery + 30 min 6% O_2). Animals were sacrificed on P10 or P45 and brains removed for immunohistochemical identification of neurons (NeuN) and CRF neurons. Counts of NeuN- and CRF-positive neurons were obtained in the CeA, BNST and PVN of control (n=8) and HI (n=8) animals for each time point. **Results:** After P3 HI there were significant losses of NeuN- and CRF-positive neurons in the CeA, BNST and PVN in P10 and P45 animals. The effects of P3 HI on CRF neuronal losses were more pronounced on P10 than P45. **Conclusions:** Results show that neurons are lost in the CeA, BNST and PVN after P3 HI and that there are specific losses of CRF-positive neurons. Neuronal losses were still apparent six weeks after HI suggesting an improvement in neuronal survival with time. However, it remains to be determined whether changes in CRF counts contribute to functional deficits apparent in the HI-affected preterm neonate.

POS-MON-108

NDFIP1 PREVENTS HUMAN NEURONAL CELL DEATH IN AN IN VITRO MODEL OF HYPOXIA

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Purpose: Previously we have shown that Ndfip1 up-regulation is correlated to neuronal survival using an *in vivo* model of rodent brain trauma. To further test Ndfip1's protective function in human neurons we have used an *in vitro* cobalt chloride model of hypoxia. **Methods:** Human cortical neurons from embryonic brains (16-18 week) were tested for the up-regulation of Ndfip1 in the presence of cobalt chloride. An inducible expression system of Ndfip1 in SH-SY5Y cells was constructed and cell death assays (both cobalt chloride and H_2O_2) were performed using FACS. **Results:** Addition of cobalt chloride to human embryonic neurons resulted in robust up-regulation of Ndfip1 (100% increase, n=3). Ndfip1 induced SH-SY5Y cells were found to have 2-3 fold protection from cell death compared to un-induced cells (n=4) in both cobalt chloride and H_2O_2 toxicity assays. Immuno-precipitation assays between endogenous Ndfip1 and the metal transporter DMT1 in human embryonic neurons revealed that DMT1 was a binding partner for Ndfip1 and this interaction led to ubiquitination of DMT1. **Conclusion:** Ndfip1 is neuroprotective against cobalt chloride treatment that induces cell death pathways similar to hypoxia (such as ROS). To further characterise the pathway of Ndfip1 protection we identified a binding partner, DMT1, which is ubiquitinated in the presence of Ndfip1. Down-regulation of DMT1 would result in reduced cobalt uptake, however this is not the sole pathway involved in Ndfip1 protection of neurons as survival is also observed under hydrogen peroxide toxicity (ROS). Importantly Ndfip1 is not a direct member of apoptotic pathways and its protection from cell death appears to function upstream of any apoptotic signals.

POS-MON-109

PROTEASOME INHIBITION: AN EARLY OR LATE EVENT IN NITRIC OXIDE-INDUCED NEURONAL DEATH?

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Purpose: Nitric oxide (NO), ubiquitously expressed in the CNS, has been perceived to be a potential neuromodulator. Employing cultured murine cortical neurons, NO indeed resulted in an inhibition of the ubiquitin-proteasome system (UPS) with a dose- and time-dependent decrease in cell viability (n=4-6). However, it is unclear whether the drop in UPS efficiency is directly imposed on by NO. **Methods:** Primary murine neocortical neurons were obtained from embryonic day 15 or 16 Swiss-white mice. The cultures were maintained in a humidified CO_2 incubator (5 % CO2, 37 ˚C) and neurons at day 7 in vitro were treated with NOC-18 (NO donor) or lactacystin (classical proteasome inhibitor) in Neurobasal medium. Fourteen Affymetrix GeneChips (34,000 well characterized mouse genes) were used and the assignment of controls/treatments: controls (n = 5), and 8 h, 15 h and 24 h NOC-18 treatments (n = 3 each group). **Results:** Our microarray analysis revealed an early down-regulation or non-significant differential expression of genes encoding UPS proteins in NOC-18 treated neurons as compared to an observed elevation of UPS corresponding genes in lactacystin treated neurons. Furthermore, time-course analysis of proteasome activity (n=4-6) in NOC18-treated neurons demonstrated a late onset of reduction (postglutamyl, chymotrypsin-like and trypsin-like peptidase activities of the proteasome). **Conclusion:** NO-triggered neuronal death takes on a different signaling cascade compared to proteasome inhibitor-mediated cell death and the late reduction of proteasome activity is a downstream event following the activation of apoptotic cellular signaling cascade.

POS-MON-110

A CELL CULTURE MODEL OF STROKE FOR THE STUDY OF TISSUE PLASMINOGEN ACTIVATOR (TPA) INDUCED DAMAGE TO BRAIN ENDOTHELIAL CELLS (BECS)

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Purpose: tPA is the only clinically approved pharmacological treatment for stroke, but it's use outside a small therapeutic window is controversial. We therefore aimed to develop an *in vitro* model of ischemia in brain endothelial cells to investigate the actions of tPA, thus establishing a high throughput screening system for the investigation of novel therapies for the treatment of stroke. **Methods:** Human BECs were cultured in various media to determine optimal growth conditions. Once confluent, BECs (n = at least 3 passages) were subsequently deprived of oxygen and glucose (OGD) for varying times (1-5 hrs) and also exposed to tPA (20 µg/ml) to mimic stroke conditions. Following OGD, lactate dehydrogenase (LDH) and tetrazolium salt (MTT) levels were analyzed to quantify cell death whilst annexin V and propidium iodide (PI) staining were performed to visualize apoptotic and necrotic cell death respectively. **Results:** BECs were eventually maintained in DMEM F-12 culture medium prior to exposure to stroke conditions. Exposure to OGD caused a time dependent change in LDH release that significantly increased after 4hrs OGD, and was exacerbated by tPA administration ($p < 0.001$). Annexin V and PI staining revealed increased apoptotic and necrotic cell death in cultures treated with OGD and tPA compared to OGD alone. **Conclusion:** These data indicate that BECs are injured during OGD, with the administration of tPA increasing the amount of apoptotic and necrotic cell death observed. Further optimization of this *in vitro* model of stroke, however, is necessary before it can be used a screening system.

POS-MON-111

PROTEIN PHOSPHATASE 1-DEPENDENT BIDIRECTIONAL SYNAPTIC PLASTICITY CONTROLS RECOVERY FROM ISCHEMIA IN THE ADULT BRAIN

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Protein kinases and phosphatases can alter the extent of excitotoxic damage resulting from ischemia by concurrently modulating apoptotic/survival pathways. Here, we show that protein phosphatase 1 (PP1), known to constrain neuronal signaling and synaptic strength (Mansuy et al., 1998; Morishita et al., 2001), critically regulates neuroprotective pathways in the adult brain. When PP1 is inhibited in the forebrain neurons, recovery from oxygen/glucose deprivation (OGD) in vitro, or ischemia in vivo is impaired. Further, in vitro, inducing LTP shortly before OGD similarly impairs recovery, an effect that correlates with strong PP1 inhibition. Conversely, inducing LTD prior to OGD elicits full recovery by preserving PP1 activity, an effect that is abolished by PP1 inhibition. The mechanisms of action of PP1 appear to be coupled with several components of apoptotic pathways, in particular ERK1/2 whose activation is increased by PP1 inhibition both in vitro and in vivo. Altogether, these results reveal that the mechanisms of recovery in the adult brain critically involve PP1, and highlight a novel physiological function for LTP and LTD in the control of brain damage and repair. (* The first two authors contributed equally to this work.).

POS-MON-112

A SYSTEMATIC REVIEW OF STEM CELL THERAPIES IN ANIMAL MODELS OF FOCAL ISCHEMIA

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Purpose: Stem cells possess the abilities both to replenish themselves and to differentiate into specialised cells including neurons, and therapies based on these properties have been proposed for the treatment of diverse neurological conditions, including stroke. For many candidate stroke treatments, efficacy in animal models has not been replicated in clinical trials. This may be due to problems both in the conduct and reporting of animal studies and/or in the design of clinical trials. A robust and systematic review of the conditions under which efficacy is observed in animals is desirable in the development of new treatments. There has been no large clinical trial of stem cell therapies in stroke; to inform the design of future trials we set out systematically to review studies reporting their use in animals. Methods: Systematic review and weighted mean difference random effects meta-analysis of studies describing the efficacy of stem cell therapy in animal models of focal ischaemia. Results: 4111 publications were identified of which 194 met our pre-specified inclusion criteria. Infarct volume was reported in 54 publications using 2012 animals and neurological score in 72 papers using 1876 animals. Infarct volume was improved by 28.9% (95% CI 24.8%-33.1%); neurological score by 34.4% (95% CI 29.5%-39.2%). Funnel plotting and Egger regression suggested a substantial publication bias. Median study quality score was 4/10 (IQR 3-5): poor quality studies reported greater improvements in reported outcome than did higher quality studies. Improvement in infarct volume was dose dependant, diminished over time and most effective in mice and hypertensive animals. Conclusions: Despite concerns about the quality of some of this literature, detailed analysis of these data will provide crucial guidance to the informed development of clinical trial protocols to test the efficacy of stem cell therapies in stroke.

POS-MON-113

CLOSED HEAD INJURED MICE ARE NOT ANXIOUS OR DEPRESSED TWO WEEKS AFTER INJURY

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Purpose: Depression is the most common mood disorder after traumatic brain injury, with rates estimated to be as high as 50%. Although, detrimental effects of depression on outcome are frequently reported (1, 2), there have been no rigorous studies either in humans or experimental animals. The aim of the present study was to examine behavioural deficits in mice subjected to closed head injury and to relate deficits to the degree of anxiety/depression measured 2 weeks post-injury. **Methods:** Briefly, C57B/6 mice (n=10) were anaesthetised with ether and subjected to closed head injury (CHI) using a weight drop device. Sham mice (n=10) were anaesthetised but not subjected to weight drop. Mice were tested 2 weeks post-injury on the elevated plus maze and in the Porsolt swim test to examine anxiety/depression. The ledged beam test was used to detect deficits in limb function. **Results:** CHI and sham mice showed no differences in the percentage of time spent in the open arms of the elevated plus maze (51 ± 16% and 63 ± 20%, respectively), or in the latency to enter the open arms (7 ± 2 s and 8 ± 3 s respectively). In the Porsolt swim test, there was no difference between CHI and sham mice in the duration of immobility (174 ± 55 s and 187 ± 59 s, respectively). **Conclusion:** These data indicate that CHI mice do not show any evidence of anxiety/depression compared with sham controls at 2 weeks following CHI. 1. Iverson, GL (2005) Curr Opin Psych 18:301-317 2. Rapoport, MJ et al (2006) J Affective Dis. 92:273-276.

POS-MON-114

CANCELLED

POS-MON-115

RELATIVELY MATURE CORTICAL NEURONS CAN WITHSTAND SIGNIFICANT PHYSICAL DISRUPTION AND ELABORATE REGENERATIVE SPROUTS

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Purpose We are interested in the capacity of neurons to respond to significant structural damage. We have developed a cell culture model involving the secondary replating of cortical neurons grown to relative maturity. **Methods** Neurons harvested from E18 Hooded-Wistar rat frontal cortex were grown in culture (Neurobasal media) until relative maturity (21 days in vitro (DIV)) on poly-L-lysine coated glass coverslips. Cortical neurons from 5 different culture preparations were then subjected to trypsinisation, mechanical scrapping and collected into a cell suspension. The neurons were then replated onto either an equivalent poly-l-lysine substrate or an astrocyte monolayer. Immunohistochemistry and live imaging were used to investigate the response of relatively mature neurons to this injury. **Results** Neurons maintained in Neurobasal media for up to 3 weeks developed dendrites, axons and synapses, and expressed neurochemical markers indicative of cellular maturity. These neurons when replated at 21 DIV were able to survive the injury and re-establish neuritic polarity, demonstrated by MAP2 and tau immunolabelling. Furthermore, live imaging indicated that the neurons were not only viable, but also had a capacity for axonal extension. Labelling with the thymidine analogue, BrdU, in combination with a neuronal marker β-III-tubulin, indicated that regenerating neurons were mature and that no additional neurogenesis had occurred. Neurons plated onto an astrocytic monolayer had a greater survival rate, and replating also induced a local astrogliosis. **Conclusions** This study indicated that mature cultured cortical neurons have the potential to survive, extend processes and re-establish polarity following significant physical damage. This new in vitro model may be useful for determining the cellular basis of neuronal structural plasticity.

POS-MON-116

AXONAL STRETCH INJURY IN AN IN VITRO CORTICAL NEURON MODEL STIMULATES AXONAL FILOPODIAL SPROUT FORMATION

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PURPOSE: The sprouting of axon collateral branches is important in the establishment and refinement of neural connections following injury. These collateral branches are initiated by localized filopodial activity along quiescent axonal shafts. In this study we examined the neuronal response to non-disruptive axonal stretch injury, particularly the stimulation of axolemmal sprouts in damaged axons. **METHOD:** Rat embryonic cortical neurons were cultured to relative maturity (21 days *in vitro*) on poly-L-lysine coated coverslips. 10nM cyclosporin-A and 0.1μM FK 506 was added two hours prior to injury to a subset of culture preparations. Axon bundles were transiently stretched to a strain level between 103-106% using controlled pressurized fluid. Live imaging of injured axonal bundles (n=12) was conducted using Mitofluor ™ green. Double immunohistochemical labelling was conducted for neurofilament-M (NFM), actin, growth associated protein-43 (GAP-43), at 24, 48, 72 and 96 hrs post-injury (PI). **RESULTS:** Limited cellular alterations involving NFM were observed at 24 hrs PI. However, by 48 hrs PI, NFM labeling was present in neuritic swellings and 'frayed' axon bundles (43%). In addition, injured mature axonal shafts developed sprout-like filopodia at 48 hrs PI, which were concentrated at regions of axonal swellings. These filopodial sprouts were labeled for GAP-43 but did not display classical 'fan-shaped' growth cone morphology when investigated using scanning electron microscopy. Cyclosporin-A and FK 506 treatment resulted in an increase in the length of these aberrant filopodial sprouts. **CONCLUSION:** These results may indicate a neuroregenerative attempt by stretch-injured axons. Calcineurin activity may play an important role in the formation of these axonal sprouts.

POS-MON-117

EXTRACELLULAR METALLOTHIONEIN INDUCES ALTERATION IN ASTROCYTE PHENOTYPE

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Metallothioneins, 6-7 kDa cystein rich metal binding proteins, are known to be expressed in astrocytes after brain injury. Metallothioneins-I & -II are known to be pro-regenerative and protective to neurons *in vitro*. There is increasing evidence to suggest that Metallothioneins-I & -II are released from astrocytes to the extracellular environment under stressful conditions. Here, it is reported that extracellular application of the metallothionein-IIA isoform (MT-IIA) can induce an astrocytic phenotype that is permissive to neurite extension *in vitro*. Treatment of astrocyte cultures with 5µg/ml MT-IIA elicited a 2-fold increase in GFAP expression ($p<0.05$, student's t-test, n=3) however, pre-treatment with the JAK/STAT signalling inhibitor AG490 was able to completely block this increase ($p<0.05$, student's t-test, n=3). Neurons seeded onto MT-IIA pre-treated astrocytes had 40% greater total neurite growth than neurons seeded onto untreated astrocytes (284.1 ± 15.4 vs 201.7 ± 12.0 µm; $p<0.01$, student's t-test, n=3) and greater length of the longest neurite per cell was observed in neurons on top of MT-IIA pre-treated astrocytes (148.5 ± 7.5 vs 115.4 ± 6.5 µm; $p<0.01$, student's t-test, n=3). This process appears independent of the ability of the cell to express intracellular metallothionein as both MT+/+ astrocytes ($P<0.05$, 1-way ANOVA, n=12) and MT-/- astrocytes ($P<0.05$, 1-way ANOVA, n=12) up-regulate the GFAP marker in response to extracellular MT-IIA treatment to a similar extent. This data provides a role for metallothionein in regulation of the CNS environment after traumatic brain injury via interaction with the JAK/STAT pathway.

POS-MON-118

NDFIP1 DIRECTS NEDD4 TO BE RELEASED IN EXOSOMES

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Ubiquitination can target proteins for degradation or change their subcellular location. Following activation, transmembrane receptors can be recognized by the ubiquitination machinery, delivered to endosomes and further to the proteasome for degradation. Nedd4 is an E3 ubiquitin ligase, involved in the traffic regulation and stability of membrane proteins. Ndfip1 (Nedd4 family interacting protein-1) is a Nedd4 adaptor protein, identified in a screen for proteins that interact with the WW domains of Nedd4. Ndfip1 was also identified in a screen for genes upregulated in the damaged cortex following traumatic brain injury (TBI). Overexpression of Ndfip1 in an in vitro model for neuronal apoptosis conferred significant neuroprotection following growth factor starvation. Ubiquitination is therefore a potential survival strategy for neurons following TBI. **Result:** Here, we show the release of Ndfip1, a transmembrane protein from primary cortical neurons in Exosomes (n=5). This has not been previously demonstrated. Exosomes are small vesicles of endosomal origin that are released into the extracellular space upon fusion of MVB (Multivesicular bodies) with the cell membrane. Release of Ndfip1 in Exosomes may serve two functions, releasing unwanted proteins and cell-cell communication. Further, we show that Ndfip1 is necessary for the secretion of Nedd4 in Exosomes (n=5). Nedd4 is only released in Exosomes when co-expressed with Ndfip1 in HEK293T cells. **Conclusion:** We propose that this represents a new pathway for the release of unwanted or damaged proteins after ubiquitination in response to Traumatic brain injury.

POS-MON-119

IMMUNE CELLS WITHIN THE SCIATIC NERVE AFTER CHRONIC CONSTRICTION INJURY IN RATS

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Purpose: Neuropathic pain after nerve injury is associated with activation of immune cells [1]. We investigated the distribution of macrophages and T-lymphocytes in the sciatic nerve above and below the lesion site, and in paw skin, after chronic constriction injury, a common model of neuropathic pain. **Methods:** Adult male rats were anaesthetized with halothane for nerve lesions and then 2-7 days later with pentobarbitone for perfusion with fixative. Different types of macrophage and T-lymphocyte were demonstrated immunohistochemically. **Results:** Immune cells started to invade the lesion site after 2-3 days (n=4), increasing markedly by 7 days (n=6). The density of CD68+/CD163+ 'resident' macrophages in the distal nerve increased ~3x compared with ~1.5x proximally. Within the lesion, and to a small extent distally, macrophages expressed CD8, implying that they were blood-derived, but CD8+ macrophages were not detected in the proximal nerve. Proximally, the density of TCR+ cells rose ~4x but CD8+ T-cells <2x, suggesting that many T-cells were CD4+. This was confirmed by the presence of CD3+/CD4+ T-cells, which were much less common than CD8+ T-cells distally. Macrophage density remained unchanged in the partially denervated dermis, although CD68+ macrophages aggregated amongst degenerating nociceptive terminals at the epidermal-dermal junction. **Conclusion:** The macrophage populations distally reflect the phagocytosis of debris as most axons degenerate below the lesion. Recruitment of CD4+ T-cells may be triggered by signals from axotomized axons whereas degeneration attracts CD8+ T-cells. CD4+ T-cells release pro-inflammatory cytokines that increase the excitability of intact unmyelinated nociceptive axons [1]. 1. Moalem G. et al., Neuroscience 129:767-7777 (2004).

POS-MON-120

PRE-CONDITIONING LESION: POSSIBILITY FOR SYSTEMIC CNS REGENERATION IN ADULT RATS

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In mammals mature spinal cord neurons do not spontaneously regenerate after injury unless the peripheral branch of the same neuron has been previously injured. This effect is referred to as 'conditioning' of the dorsal root ganglion (DRG) neurons. Previous work from our laboratory revealed 3 important findings observed from this type of injury. Firstly, a peripheral pre-conditioning lesion led not only to CNS regeneration of ascending fibers but also descending fibers through the injury epicentre. Secondly, this type of CNS regeneration could be enhanced by vaccination with sciatic nerve homogenate in neonates and adult rats. Finally, pre-conception vaccination with sciatic nerve homogenate induced tolerance, thereby suppressing spontaneous regeneration triggered by the pre-conditioning lesion. **Purpose:** based on these results we hypothesised that the immune response induced by a peripheral nerve injury may have a 'systemic effect' on CNS regeneration. **Methods:** surgically under Fluorothane anaesthesia, peripheral nerve injury was performed as previous, however in this occasion instead of injuring that same branch as part of the spinal cord injury complex, the location of the CNS lesion was changed to the optic nerve. Adult Sprague Dawley rats were divided into 3 groups (n=5 per group). Group (A - Test) received a sciatic nerve lesion followed by an optic nerve crush; Group (B – Sham) received a sciatic nerve exposure plus an optic nerve crush; and Group (C – Control) received an optic nerve crush only. **Results:** a peripheral pre-conditioning lesion did not exert a systemic regenerative response, therefore, there must be a local trigger for the enhanced regeneration observed in original pre-conditioning injury model. **Conclusion:** this study presents evidence for possible local mechanism in the pre-conditioning lesion model.

POS-MON-121

PROTEOMIC ANALYSIS OF THE SPINAL CORD FOLLOWING INJURY IN THE DEVELOPING OPOSSUM

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The immature mammalian spinal cord, unlike the adult, is capable of substantial morphological repair following injury. Analysis of developmental changes in response to spinal injury could lead to better understanding the limited repair in adults. A marsupial, the South American opossum, was used in this study. The opossum genome has recently been published (Mikkelsen et al, Nature, 2007). **Purpose:** To use differential proteomics to identify developmental changes in proteins in the spinal cord in response to complete spinal transection. **Methods**: Four groups of opossums were used: (i) injured at postnatal day (P) 7 and collected at P14; (ii) control P14; (iii) injured at P14 and collected at P21; and (iv) control P21. Spinal cord transection at T10 was performed under anaesthesia. Cords from whole litters (6-8 pups) were pooled to yield sufficient tissue. Two-dimensional (2D) gel electrophoresis was used to separate proteins based on isoelectric point and subunit molecular weight and allowed analysis of the whole protein extracts of spinal cord segments. Differential protein 'spots of interest' were identified by computer-aided overlaying of gel images. Mass spectrometry was used to identify proteins of interest. **Results:** 2D gels have been generated for all groups. While all groups express a large amount of homologous proteins, small differences in the proteomic profiles have been identified. Preliminary data included two protein spots of interest that were identified by mass spectrometry as enolase (74kD) and transportin 1 (103kD). **Conclusion:** We identified several changes in the protein population in spinal cord tissue during development that may reflect the differential ability of the cord to repair following injury.

POS-MON-122

THE THERAPEUTIC EFFECT OF LIF ON AXONAL INJURY DURING EAE-ASSOCIATED OPTIC NEURITIS

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The pathology of the autoimmune inflammatory demyelinating disease multiple sclerosis (MS) also features inflammatory axonal degeneration within the brain. Cumulative axonal degeneration is the likely cause of the majority of progressive MS-related disability, and therefore, the need for novel neuroprotective therapies for MS is apparent. Experimental autoimmune encephalomyelitis (EAE), a long used model for aspects of MS pathology, also produces axonal injury, the optic nerve (ON) being a key site of pathology for mouse EAE. **Purpose:** The effects of the putative neuroprotective cytokine, leukaemia inhibitory factor (LIF) were investigated in the optic nerve by obtaining a number of outcome measures targeting axonal dysfunction. **Methods:** MRI measures of water diffusivity along (ADC ||) and across (ADC$^{\perp}$) ONs, blood plasma levels of phosphorylated neurofilament heavy chain subunit (pNF-H) and histological morphometric measures of ONs were obtained from EAE-induced mice treated with either placebo or LIF. **Results:** LIF treatment reduced EAE grade (n>12 per group) and pNF-H blood plasma levels (n>7 per group), restored ADC$^{\perp}$ (n>38 per group) and improved cross-sectional axolemmal area (n>7 per group), while had no effect on ADC || (n>38 per group) and other histological parameters, such as axon counts or inflammatory infiltration (n=7 per group for histological measures). **Conclusion:** These results suggest that the therapeutic effect of LIF may be mediated by the axon or neuron.

POS-MON-123

MURINE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AS A MODEL OF AXONAL PATHOLOGY IN ACTIVE MULTIPLE SCLEROSIS LESIONS

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Purpose: Axonal injury in active multiple sclerosis lesions represents an important therapeutic target. To facilitate therapeutic trials of neuroprotective candidates, we aimed to validate murine experimental autoimmune encephalomyelitis (EAE) as a model of acute inflammatory axonal injury using a novel surrogate marker of axonal degeneration, phosphorylated neurofilament H (pNF-H). **Methods**: MOG_{35-55} EAE was induced in C57BL/6 mice and disease severity was assessed daily. Blood samples were collected from the tail veins at EAE days 12 and 22 (n=27), 35 (n=8) and 50 (n=17) for assessments of axonal injury by pNF-H ELISA. Axonal number and levels of inflammation were assessed in lumbar spinal cord sections. **Results**: Serum pNF-H levels were elevated at EAE day 22 relative to healthy control mice (23.4±2.41 vs 0±0.5ng/ml), and strongly correlated with disease severity scores (R=0.7480; P<0.0001). Negligible serum pNF-H levels were detected at EAE days 35 and 55 (0.004±0.003ng/ml and 0.06±0.03ng/ml), suggesting this is a model of acute axonal injury. At EAE day 22, serum pNF-H levels were found to strongly correlate with axonal loss in the dorsal column (R=-0.8050;P<0.001), validating serum pNF-H levels as a surrogate marker of axonal loss. It was subsequently shown that serum pNF-H levels were associated with inflammation in the spinal cord (R=0.7446; P=0.0055), and that axonal injury could be reduced following treatment with an anti-inflammatory drug. **Conclusions**: These findings validate serum pNF-H levels as a surrogate marker of axonal injury, and show that C57BL/6 MOG_{35-55} EAE is likely to model acute inflammatory axonal injury.

POS-MON-124

BONE MORPHOGENIC PROTEIN MODULATES THE NEURAL PRECURSOR CELL RESPONSE TO CENTRAL DEMYELINATION

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Purpose: Our aim is to determine the effects of modulation of bone morphogenic protein (BMP) signaling during myelin injury in adult mice. BMPs are important inhibitory signals for oligodendrogenesis and have been shown to decrease neural precursor cell (NPC) proliferation and production of oligodendrocytes in early development, however the regulatory role of BMPs in the injured adult brain is not known. We previously reported that BMP4 and BMP receptors are upregulated in the subventricular zone (SVZ) during myelin injury. **Methods:** NPCs were isolated from the SVZ of adult mice with or without cuprizone-induced central demyelination. Proliferation and differentiation assays were performed in the presence or absence of BMP or noggin (a BMP inhibitor) and immunostained for BrdU or lineage specific markers. **Results:** Here we report that BMPs decrease NPC proliferation (control=8.7%, BMP2=0.4%, BMP4=0.3%), increase commitment to glial fibrillary acidic protein positive (GFAP+) astrocytes (control=52.3%, BMP2=93.8%) and decrease oligodendrocytes (control=36%, BMP2=5.6%) in adult SVZ-derived NPCs (n=3). Furthermore, we demonstrate that BMP4 causes an increased in production of GFAP+ cells by NPCs from myelin-injured animals as compared to control animals (n=4, 4) and application of Noggin (a BMP inhibitor) causes an increase in proliferation of NPCs from myelin-injured animals compared to controls (n=4,4). **Conclusion:** This supports a model where activation of BMP signaling in NPCs by myelin injury inhibits proliferation and increases the production of astrocytes by NPCs and inhibiting BMP signaling increases proliferation, reduces astrocyte production and increase oligodendrogenesis to enhance repair. We are currently examining the timecourse of activation of the BMP signaling pathway and further characterizing the effects of BMP4 and Noggin on NPCs during demyelination.

POS-MON-125

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

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Purpose: An early feature of many multiple sclerosis lesions is oligodendrocyte apoptosis. To study the neurobiology of such lesions and subsequent repair, a non-immune mediated model of oligodendrocyte apoptosis is required that is easily inducible, non strain-dependent, and ablates mature oligodendrocytes in a consistent, well-characterised manner. Here we describe progress towards generating such a model, utilising a transgenic approach in which mature, myelin basic protein (MBP)-expressing oligodendrocytes are induced to express the Diphtheria Toxin receptor (DTR), rendering them selectively vulnerable to Diphtheria Toxin (DT). **Methods:** Firstly, the MBP-DTR construct, comprising a proximal murine MBP promoter and DTR coding sequence was cloned, and its efficacy validated in an in vitro system using the CG4 oligodendrocyte progenitor cell line. Next, the MBP-DTR fragment was microinjected into fertilised C57BL/6 eggs to generate transgenic founders. For each line generated, specificity of DTR expression to the mature oligodendrocyte population is currently being assessed. **Results:** DT administration to CG4 cells transiently transfected with the MBP-DTR construct significantly reduced cell viability over doses ranging from 10ng/ml-10000ng/ml (*p<0.05*; n=3). The microinjection procedure successfully generated twenty-six MBP-DTR founders, with 70% transmitting the transgene to offspring. Thus far two MBP-DTR lines have been assessed for DTR expression; both express the DTR in a small percentage of cells that morphologically resemble mature oligodendrocytes and colocalise with oligodendrocyte-specific markers. **Conclusion:** The MBP-DTR construct described here could be of significant value in understanding the molecular and cellular responses to demyelination following oligodendrocyte apoptosis, and for defining new strategies to enhance remyelination and repair.

POS-MON-126

DIFFUSION TENSOR ANALYSIS OF OPTIC RADIATION CHANGES AFTER OPTIC NEURITIS

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Purpose: Optic neuritis (ON) results from optic nerve pathology and is a common presentation of Multiple Sclerosis. ON patients show abnormal visual function measured using visual evoked potentials (VEP), and changes in optic nerve diffusivity measured using diffusion MRI. VEP and optic nerve diffusivity changes correlate moderately, however optic radiation lesions could also contribute to changes in visual function. This study aimed to compare mean diffusivity (MD) in the optic radiation between control and patient normal-appearing white matter (NAWM), and between patient NAWM and lesions. **Methods:** Eight healthy control subjects (7f/1m; age = 35.4 ± 11.9yrs) and fifteen ON patients (12f/3m; age = 34.8 ± 8.0yrs; symptom onset = 4.0 ± 0.4yrs) were scanned using 54 direction DTI and a FLAIR sequence for lesion identification. Probabilistic tractography was used to define optic radiation regions of interest (ROI) bilaterally. Patient ROIs were manually segmented into NAWM and lesions according to areas of hyperintensity on FLAIR scans. MD was calculated within each ROI and control and patient ROIs were compared using Student's t-tests. **Results:** MD was significantly higher in patient NAWM compared to controls (patient NAWM: 806 ± 24 $x10^{-6}mm^2s^{-1}$; control: 791 ± 17; p=0.02). Optic radiation lesions were found in 10 patients contributing 4.8 ± 4.5% of tract volume in these patients. MD was significantly higher in lesions compared to NAWM (lesion: 988 ± 113 $x10^{-6}mm^2s^{-1}$; p<0.001). **Conclusions:** ON patients' optic radiation MD was significantly increased compared to controls', resulting from both lesion activity and changes in NAWM.

POS-MON-127

ENDOGENOUS BRAIN-DERIVED NEUROTROPHIC FACTOR MEDIATES ASCENDING TRACT REGENERATION INTO THE INJURED SPINAL CORD FOLLOWING A CONDITIONING SELECTIVE MOTOR NERVE INJURY

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Lumbar 5 ventral root transection (L5 VRT) induces neuropathic pain and triggers upregulation of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). We hypothesize that VRT could enhance the regeneration of injured ascending sensory neurons. Sprague Dawley rats, anaesthetized by Isofluorane, were subjected for L5 VRT one week earlier and then for the dorsal column cut. Regenerating neurons were retrogradely traced by Fast Blue. After L5 VRT, BDNF mRNA and protein are highly expressed in the dorsal horn, motor neurons of the spinal cord and dorsal root ganglia (DRG). Ventral root transection resulted in a significant number of Fast Blue+ neurons in the ipsilateral DRG after dorsal column cut. This combined injury increased BDNF-like immunoreactivity in the dorsal column caudal to the lesion site and increased the expression of p75NTR in the glia but had no effect on the expression of p75NTR in sensory neurons in the dorsal root ganglia. Most regenerating sensory neurons are surrounded by p75+ glia and express BDNF and trkB but not p75NTR, indicating physiological overexpression of BDNF in sensory neurons overrides the p75NTR-mediated inhibitory signals. Taken together, L5 VRT can promote regeneration of dorsal root into the spinal cord, most likely by increasing BDNF levels in the spinal cord and DRG and by overriding the activation of neuronal p75NTR.

POS-MON-128

ROLES OF PROBDNF IN RAT RETINA FOLLOWING ACUTE HIGH INTRAOCULAR PRESSURE

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Glaucoma causes degeneration of retinal ganglion neurons and loss of vision and mature brain derived neurotrophic factor (BDNF) can prevent retinal degeneration in animals with high intraocular pressure (HIOP). Pro-neurotrophins may have different functions from mature forms during development and after nerve injury. In the present study, we investigated the role of endogenous proBDNF on rat retina following HIOP. Rats, anaesthetized by pentobarbital, were subjected for HIOP by infusion of saline into the anterior chamber to keep intraopcular pressure at 110 mmHg for 60 min. Two days before HIOP, rats received an injection of proBDNF antibody, mature BDNF antibody, proBDNF, mature BDNF or control IgG only. The receptors of proBDNF, P75NTR and Sortilin were detected. And retinas were examined at various time points by staining of retinal ganglion cells, astrocytes and macrophages. P75NTR immuno positive products were found in the plexiform layers of retina, while Sortilin was mainly located in cells of the ganglion cell layer. Compared to control IgG anti-proBDNF pretreatment inreased cell number in the ganglion pretreatment, cell layer after HIOP injury. At the same time, expression of ED1 and GFAP was remarkably up-regulated with anti-proBDNF pretreatment after HIOP, indicating that macrophage invasion and glia activation may be involved in the function of anti-proBDNF after HIOP injury. The data suggest that endogenous proBDNF may have detrimental effects after nerve injury and the antibody to proBDNF may have therapeutic effects in acute glaucoma.